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Biological Activities of Purified Marennine, the Blue Pigment Responsible for the Greening of Oysters

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ABSTRACT: Marennine, the blue pigment produced by the diatom *Haslea ostrearia*, exists in two different forms, the intra- and extracellular forms. We investigated the antibacterial, antiviral, and antiproliferative properties of both of these forms. Both forms of marennine inhibited the development of marine bacteria, in particular the pathogenic organism *Vibrio aesturianus*, at concentrations as low as 1 μg/mL, but they did not display any effect on a wide range of pathogenic bacteria that are relevant for food safety. Both forms of the pigment produced by *H. ostrearia* also exhibited antiviral activity against the HSV1 herpes virus, with intra- and extracellular marennine having EC_{50} values of 24.0 and 27.0 μg/mL, respectively. These values are 2 orders of magnitude higher than the value for the reference drug, Zovirax. Moreover, both forms of marennine were effective in slowing or inhibiting the proliferation of cancer cells. This study confirms the potential of marennine as a biologically active organic molecule, which could have a protective effect on bivalves, which filter seawater and fix the pigment on their gills. Moreover, marennine could be used in food engineering and chemistry as a natural blue pigment. However, despite that it is eaten and possibly assimilated by green oyster consumers, it also deserves in depth evaluation before being considered for use as a nutraceutical.

KEYWORDS: *Antibacterial, antiproliferative, antiviral, green oysters, Haslea ostrearia, marennine*

INTRODUCTION

Haslea ostrearia is a pennate diatom, thought to be distributed worldwide, which produces a water-soluble blue pigment known as "marennine" (or marennin).[1] This name refers to the Marennes-Oléron Bay, which has been an area of intensive oyster farming since the end of the 18th century and which is now the most important oyster-rearing area in Europe. Before being marketed, some of the oysters from the Marennes-Oléron Bay undergo a fattening process known as "affinage", which mostly takes place in ponds, known as "claires", located in ancient salt marshes.[2] The duration of the fattening period and the density of the oysters in the ponds are regulated according to specific standards.[3] In some ponds, *H. ostrearia* can outcompete other microalgae and become seasonally dominant, a phenomenon responsible for the "greening" of the ponds and, as a consequence, of the oysters. Indeed, marennine released into the seawater during *H. ostrearia* blooms is filtered by the oysters and remains on their gills and labial palps, giving them a specific and unusual green color. This phenomenon has long been known and was first referred to in England.[4]

A second consequence is that marennine has been indirectly consumed by some human beings for centuries. Indeed, although "green oysters" (known as "vertes de claires" in France) cost about 20% more than ordinary fattened oysters (known as "fines de claires"), they are renowned and are traditionally eaten during festive periods, which has contributed to the economy and reputation of local oyster production in the Marennes-Oléron Bay. Greening of oyster gills is a reversible phenomenon, and it is supposedly concomitant with changes in the organoleptic properties of the oysters,[5] although no direct role of marennine has been identified,[6] except for the change in color. For decades, the chemical nature of marennine remained unknown, and many possibilities were suggested, such as its being an anthocyanin, chromoprotein, or chlorophyll degradation product.[7] Our knowledge of the chemical nature of marennine was recently increased when a method for its extraction and purification was published.[8] Preliminary characterization suggests that marennine is related to a non-hydrolyzable polyphenol of medium molecular weight (ca. 10 kDa),[9] but its chemical structure has not yet been established. Two forms of the pigment have been described: one is intracellular (IMn), accumulating mainly at the apical regions of the algal cells, and the other is extracellular (EMn), with the

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latter being released into the culture medium. It has been demonstrated that the IMn and EMn forms of marennine differ with regard to both their molecular weight and spectroscopic characteristics.[8]

Both forms of purified marennine have been shown to display allelopathic[10] and antioxidant properties.[11] Moreover, antiproliferative and antitumor activities,[12] as well as antiviral and anticoagulant activities,[13] have been observed when aqueous crude extracts of *H. ostrearia* were tested. All of these types of biological activity have been recorded with various marine polyphenols,[14] which is consistent with the probable polyphenolic nature of marennine.[15] Hence, the two goals of this work were to further confirm the antiviral, antiproliferative, and antitumor activities previously observed using crude extracts of *H. ostrearia*, using now purified marennine, and to extend our knowledge of the biological activities of this peculiar blue pigment by testing its activity against bacteria, which are a major component in the microfouling phenomenon and an important factor for food quality.

■ MATERIALS AND METHODS

Cultivation of Algae and Biomass Collection. The different strains of *H. ostrearia* used for biomass production and pigment extraction were provided by the Nantes Culture Collection (NCC, University of Nantes). They were derived from samples collected in Bourgneuf Bay, France (46° 59′ 19″ N/2° 14′ 14″ W). Algae used to test antiproliferative and antitumor activities were grown in modified Provasoli medium (ES Provasoli 1/3) at 16 °C at an irradiance of 100 μmol of photon m^{-2} s^{-1}, with illumination provided by cool-white fluorescent tubes in a 14/10 h light/dark (L/D) cycle.[16] The first culture was performed in a 250 mL Erlenmeyer flask and then was used to inoculate a 2 L flask, which was, in turn, used to inoculate a 22 L flask under the same conditions.

For antifouling and antiviral analyses, the algae were cultured in Erlenmeyer flasks containing modified artificial seawater[17] under controlled conditions (15 ± 1 °C, irradiance of 200 μmol of photon $^{-2}$ s^{-1}, and 14/10 h L/D cycle). The culture cabinet was illuminated by Philips TLD 36W/965 fluorescent tubes. The irradiance was measured using a Li-Cor LI-189 quantum meter coupled with a 2Π Li-Cor Q21284 quantum sensor. Cultures were harvested regularly during their exponential phase of growth. The cells and supernatant of all of the cultures were separated by centrifuging using a Sigma 3K15 centrifuge (Bioblock Scientific). Freeze-dried cells were stored in a freezer at −20 °C until extraction. The supernatants were stored in the dark in a temperature-controlled room at 6 ± 1 °C until analysis.

Pigment Extraction and Purification. After centrifugation, pigments were extracted and purified as described elsewhere.[8] Cells were crushed with a mortar and pestle in 250 mM NH_4HCO_3 buffer solution (pH 8) in liquid nitrogen. The crude extract containing the internal form was then centrifuged, and the supernatant was collected. For the external form of the pigment, 20 L of colored culture supernatant were used directly after filtering over a 0.45 μm filter. Then, for both extracts, a two-step ultrafiltration process was performed. Cartridges fitted with regenerated cellulose spiral membranes (Prep/Scale-TFF cartridge 0.23 m^2, Millipore) were used, first with a 30 kDa cutoff membrane and then with a 3 kDa cutoff membrane. At the end of the process, the retentate was collected. The pigment was then separated by anion-exchange chromatography, using an ultraviolet−visible (UV−vis) detector to identify the fraction on the basis of its spectroscopic properties. Purity was established by high-performance liquid chromatography−diode array detector (HPLC−DAD) control (exclusion diffusion, anion exchange).[8] The fraction collected was dialyzed and then freeze-dried. Purified pigments were stored in the dark at −20 °C.

Antibacterial Activities. Three marine bacterial strains, *Polaribacter irgensii* (ATCC 700398), *Pseudoalteromonas elyakowii* (ATCC 700519), and *Vibrio aesturianus* (ATCC 35048), were grown in steam-sterilized seawater mixed with peptone (final concentration of 5 g/L), in an incubator at 25 °C, and regularly subcultured with fresh medium. Antibacterial testing of marennine was performed by the disk diffusion technique in agar-plated Petri dishes.[18] Whatman filter paper discs 6 mm in diameter were sterilized at 15 × 10^5 Pa for 15 min. They were loaded with 10 μL of an IMn or EMn marennine solution at concentrations of 1, 10, 50, and 100 μg/mL. They were then dried in an axenic place at room temperature for 6 h. A dilution of the bacterial culture in fresh medium was made 1 day before the subsequent experiments, and then 0.1 mL samples of the culture [10^6 colony forming units (CFU)/mL] were used to seed the agar plates. After incubation for 2 days at 25 °C, the activity was evaluated by measuring the radius of the inhibition ring around the discs. All inhibition assays were carried out in triplicate.

Pathogenic Bacteria. The following strains of terrestrial bacteria were also used to test for possible antibacterial activity: *Proteus mirabilis* (Laboratoire Départemental de la Sarthe), *Bacillus cereus* (ATCC 10876), *Listeria monocytogenes* (ATCC 19115), *Clostridium perfringens* (ATCC 13124), *Campylobacter jejuni* (ATCC 33291), *Pseudomonas aeruginosa* (ATCC 104009), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 14028), and *Enterococcus faecalis* (ATCC 29212). Cultures in the exponential growth phase were diluted to the McFarland concentration conventionally used to carry out antibiograms, using a Densimat densitometer (Biomérieux), and sterile flasks of aqueous 5% NaCl solution (Biomérieux). Petri plates containing Mueller Hinton 2 agar with 5% sheep's blood were inoculated with 1 mL of a diluted suspension of bacteria. Sterile Whatman filter paper discs 6 mm in diameter, prepared as above with 10 μL of IMn or EMn solution at four different concentrations (1, 10, 50, and 100 μg/mL), were added to the Petri plates. The plates were then placed in a culture cabinet for 2 days (Fisher Bioblock Incucell cabinet for bacteria) and cultured in a normal atmosphere at 37 or 41 °C (for *C. jejuni*) and in Galaxy 170S from New Brunswick Eppendorf for *E. faecalis* and *S. aureus* grown with CO_2. *C. jejuni* was cultured under microaerophilic conditions using a CampyGen CN0020C kit from Oxoid, and *C. perfringens* was cultured under anaerobic conditions using an Anaerogen compact AN0010C kit from Oxoid. After incubation overnight, the diameters of the inhibition rings around the discs were measured. All inhibition assays were performed in triplicate.

Antiviral Activities. African green monkey kidney cells, Vero cell line ATCC CCL81, were grown in Eagle's minimum essential medium (MEM, Laboratory Eurobio) supplemented with 8% fetal calf serum (FCS), to which was added 1% PCS (10 000 units of penicillin, 25 000 units of colimycin, and 10 mg of streptomycin). Cells were routinely passaged every 3 days. A virus stock of *Herpes simplex* virus type 1 (HSV-1), wild 17 strain ACV^S, and PFA^S was obtained from Prof. Billaudel, Laboratoire de Virologie de Nantes (France). Subsamples of the virus stocks were prepared by incubating Vero monolayers (75 cm^2 culture flasks seeded with 350 000 cells/mL) at low multiplicity and incubating at 37 °C in a 95% air/5% CO_2 (v/v) atmosphere. At 2 or 3 days after infection, the cultures were frozen and thawed twice, before clearing the preparation by centrifuging at low speed to remove cell debris. The resulting supernatant aliquot was stored at −70 °C until use. Virus titrations were performed by the Reed and Muench dilution method,[19] using 10 wells of 96-well microtiter plates per dilution. The virus titer was estimated from the cytopathogenicity and expressed as the 50% infectious dose per milliliter (ID_{50}/mL). For use as an antiviral reference drug, the sodium salt of Zovirax IV, 25 mg/mL, was purchased from the Wellcome Foundation Ltd.

Dilutions of Zovirax and pigments (50 μL aliquots) were prepared in Eagle's MEM supplemented with 8% FCS and distributed into the wells of a 96-well microtest III tissue culture plate (Nunclon, Intermed). Each series included eight assays ranging from concentrations of the pigments of 200, 50, 10, 5, and 1 μg/mL (4 wells per concentration). A total of 100 μL of cellular suspension (3.5 × 10^5 Vero cells/mL) in Eagle's MEM containing 8% FCS was then distributed into the wells using a multichannel Titertek pipet and infected with HSV-1. A total of 50 μL of mock- and virus-infected cell

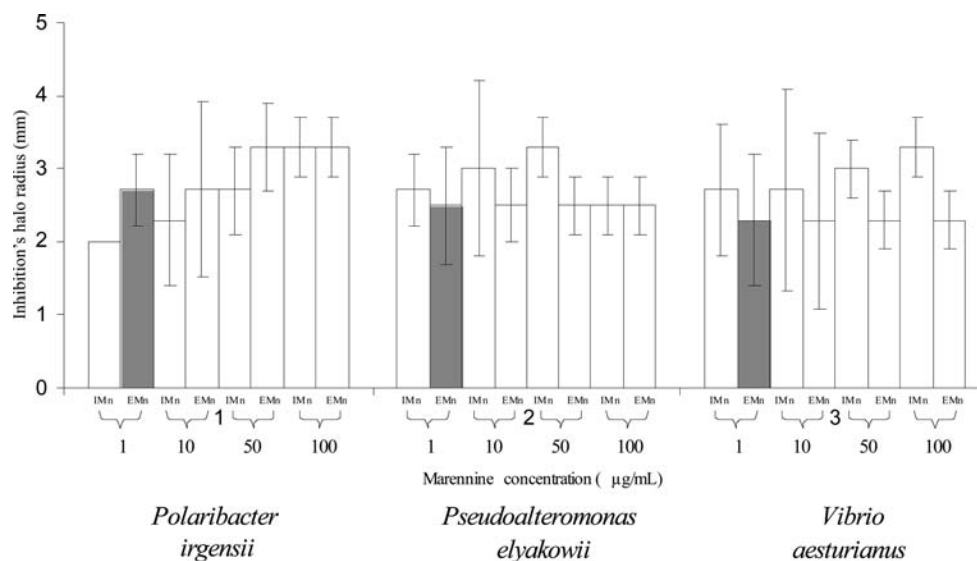

Figure 1. Antibacterial activities of purified IMn and EMn marennine on marine bacteria are expressed in millimeters of the inhibition radius [mean ± standard error (SE); $n = 3$] around the loaded paper discs. IMn, intracellular marennine; EMn, extracellular marennine.

suspensions at a multiplicity of infection (MOI) of 0.001 ID_{50}/cells ($2 \times 10^{6.5}$ ID_{50}/mL) were then added to each well containing the diluted compound and were then incubated at 37 °C with 5% CO_2 for 3 days without changing the medium. Cells and virus controls were run simultaneously.

After microscopic examination to check for viral growth, 50 μL of neutral red dye (0.15% in saline at pH 5.5) was added to each well and the cultures were incubated for 45 min at 37 °C.[20] Excess dye was removed by rinsing with phosphate-buffered saline (PBS at pH 7.2, Biomérieux), and the neutral red incorporated by the viable cells was eluted into 100 μL/well of citrate ethanol buffer. After the tray was shaken for 20 min, to completely disrupt the cell monolayers, the absorbance (OD) of the wells was read in a multichannel spectrophotometer (Packard Spectra Count) at 540 nm. The OD was directly related to the percentage of viable cells, which was, in turn, inversely proportional to the cytopathic effect (CPE) ratio. The regression slope was determined for each assay and for each plate on the basis of the cell controls (0% CPE) and virus controls (100% CPE).[21] The 50% cytotoxic concentration (CC_{50}) of the test pigment was defined as the concentration of marennine that reduced the absorbance of mock-infected cells to 50% of that of the controls. The 50% antiviral effective concentration (EC_{50}) was expressed as the concentration that provided 50% protection of the virus-infected cells against the HSV-induced destruction. The percentage protection was calculated using the following formula:

$$[(\mathrm{ODt})\mathrm{HSV} - (\mathrm{ODc})\mathrm{HSV}]/[(\mathrm{ODc})\mathrm{MOCK} - (\mathrm{ODc})\mathrm{HSV}] \times 100\ (\%)$$

where (ODt)HSV is the absorbance of the test sample (with marennine), (ODc)HSV is the absorbance of the virus-infected control (no marennine), and (ODc)MOCK is the absorbance of the mock-infected control. The ratio (ODc)HSV/(ODc)MOCK is expressed as a percentage of the control value.

Antiproliferative Activities. Internal and external marennine were solubilized in RPMI 1640 medium at 1 mg/mL. Solutions were sterilized by filtering over a 0.2 μm filter (RC-Millipore). Dilutions were conducted in PBS medium under axenic conditions, to obtain concentrations of 1, 10, 50, and 100 μg/mL. The cell lineages used were derived from human tumor lineages: M113 is from melanoma cells; SKOV3 and SHIN3 are from ovarian cancer cells; SW116 is from colon; R3111 is from kidneys; 1355 is from lungs; and MCF7 is from breast cancer cells. They were maintained at the INSERM U463 facility (University of Nantes). Another lineage of human bronchopulmonary cells (NSCLC-N6) was kept in the Marine Pharmacological Laboratory of the University of Nantes.

Cultures were carried out on RPMI 1640 medium containing 5% fetal bovine serum, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mM glutamine. Cultures were placed in an incubator at 37 °C in a CO_2-enriched atmosphere at 5%. Regular refreshment of the medium kept the cultures in the exponential growth phase. An optical method was used to estimate cellular growth by a viability test based on the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into formazan by the mitochondria of living cells.[22] Sterile 96-well cell culture plates were incubated with 50 μL of cell culture in the exponential growth phase. The 50 μL aliquots of marennine solutions or medium were then added. Some wells were filled with pure medium or only marennine solutions and used to determine the blank settlement.

The absorbance of the wells was checked on the first day using a Titertek Multiscan MKII microplate reader at 570 nm ($A_{d0[0]}$). After 72 h, 50 μL of MTT (Sigma) dissolved in phosphate buffer saline at 2.5 mg/mL was added in each well. After incubation for 4 h at 37 °C, 100 μL of a lysate buffer made with 12.5% sodium dodecyl sulfate (SDS), 30% *N*,*N*-dimethylformamide, 2.4 mM HCl, and 1% acetic acid was added to each well. After incubation for 12 h at room temperature, a new reading of the absorbance at 570 nm was performed on the text sample ($A_{d3[Mn]}$) or the growth control without marennine ($A_{d3[0]}$). The absorbance found in the assays with marennine was corrected by the marennine control recorded on day 3 without cells ($A_{d3[Mn]c}$), and the percentage growth (G%) of the control was determined as follows:

$$G\% = \{(A_{d3[Mn]} - A_{d3[Mn]c} - A_{d0[0]})/(A_{d3[0]} - A_{d0[0]})\} \times 100$$

This was used to determine IC_{50}, the minimum inhibitory concentration of marennine required to cause 50% growth inhibition compared to the control. IC_{50} values, obtained by plotting the inhibition rate ($I\% = 1 - G\%$) versus the marennine concentration, were calculated using a sigmoid dose−response model with a four-variable slope from the dose−response curve. Statistical analyses were applied at a maximum significance level of 5% by one-way analyses of variance (ANOVA), followed by a Student−Newman−Keuls (SNK) posthoc multicomparison test. Data were computed using the software SigmaPlot, version 9.0, for Windows.

RESULTS

Antibacterial Activities. Both the IMn and EMn forms of marennine significantly inhibited the growth of marine bacteria around the paper discs. The diameter of the inhibition ring generally increased with the concentrations used to load the discs. Concentrations as low as 1 μg/mL produced inhibition rings (Figure 1). No inhibition zone was observed in any culture of terrestrial pathogenic bacteria for any form or concentration of marennine (not shown).

Antiviral Activity and Cytotoxicity. After 3 days of treatment, microscopically visible changes in the normal cell morphology were observed and viability assays revealed the destruction of cell layers. No cytotoxic effect of the compounds on the Vero cells was observed with the EMn form of the pigment in the range of the concentrations assayed (Table 1).

Table 1. Antiviral Activities of Purified IMn and EMn Marennine

code	CC_{50}[a] (μg/mL)	EC_{50}[b] (μg/mL)
Zovirax	>200.0	0.2
IMn marennine	107.2	24.0
EMn marennine	>200.0	27.0

[a]CC_{50} is the 50% cytotoxic concentration, the concentration that reduced the absorbance of mock-infected cells to 50% of that of controls. [b]EC_{50} is the 50% antiviral effective concentration, the concentration that achieved 50% protection of virus-infected cells from HSV-induced destruction.

Both the EMn and IMn forms of marennine displayed effective antiherpetic activity with EC_{50} values of 24–27 μg/mL, 2 orders of magnitude higher than that of Zovirax, the reference drug. For a MOI of 0.001 ID_{50}/cells, 100% cellular protection was obtained with 200 μg/mL EMn and IMn pigment extracts 72 h after infection.

Antiproliferative Activity. The morphology of cells incubated with the IMn or EMn form of marennine differed from that of the controls. Cells no longer adhered to the plates and showed a globular shape. They grew in piles, floating in the medium. Only the SHIN 3 lineage, which was not initially adhesive, did not display any change in shape. During the experiments, no degeneration or toxicity was observed in any of the cultures (no reversal of growth).

The inhibition of growth depended upon the cell lines concerned (Table 2). For instance, the non-adherent SHIN3 cell line was insensitive to both forms of the pigment at all of the doses tested. The proliferation of cell lines SKOV-3, SW116, and M113 was inhibited at high concentrations (100 μg/mL, 31 ± 9%, 36 ± 4%, and 49 ± 3%, respectively) by EMn but only that of SW116 by IMn (100 μg/mL, 38 ± 7%). The antiproliferative activities, as illustrated by the IC_{50} values, were between 0.79 and 82.7 μg/mL for IMn and between 1.10 and 25.9 μg/mL for EMn (Table 2). For cell lines 1355, MCF-7, and R3III, for which IC_{50} were determined with both EMn and IMn, IC_{50} values were not significantly different between the two forms of marennine (SNK; $p < 0.05$), mainly because of high variability between the six replicates.

DISCUSSION

With respect to algae and human interests, marennine, the blue pigment responsible for the greening of oysters, constitutes a real paradox. On the one hand, it was first named officially 2 centuries ago[1] but may have been consumed for much longer than that, and on the other hand, its chemical structure has not yet been elucidated, although a preliminary characterization has recently been achieved and a hypothetical polyphenol nature has been suggested.[9] Furthermore, aqueous blue extracts of *H. ostrearia* containing marennine have been shown to exhibit some biological activities,[12,13] but it was impossible to identify the role of marennine before a method of extraction and purification became available. Such a method has now been developed,[8] and the study reported here was intended to investigate various potentially valuable biological activities of marennine, in addition to its known allelopathic[10] and antioxidant properties.[11]

Table 2. Antiproliferative Activities of Purified IMn and EMn Marennine on Human Cancer Cell Lines[a]

cell line		IC_{50}[b] (μg/mL ± SE) EMn	IMn
lung cancer	1355	1.10 ± 0.56 d	0.79 ± 0.35 d
lung cancer	NSCLC-N6	14.4 ± 9.8 cd	nd[c]
breast canceer	MCF-7	6.5 ± 3.5 cd	22.2 ± 8.7 bc
kidney cancer	R3III	25.9 ± 9.8 bc	36.7 ± 1.1 b
melanoma	M113	>100	82.7 ± 33.0 a
ovarian cancer	SKOV-3	>100	no[d]
ovarian cancer	SHIN3	no	no
colon cancer	SW116	>100	>100

[a]Statistical analyses were performed using all IC_{50} determined. Values are the mean ± SE for $n = 6$. Different letters next to each value indicate significant group difference (ANOVA, SNK; $p < 0.05$). [b]IC_{50} is the minimum inhibitory concentration of marennine necessary to cause 50% inhibition of growth compared to the control. [c]nd = not determined. [d]no = no inhibition observed.

Antibacterial Activities. The IMn and EMn forms of marennine inhibited the growth of the three strains of marine bacteria tested, a finding that is relevant for aquaculture (e.g., for stock production or controlling the interactions between microorganisms and fauna in oyster ponds) and the food industry. Some species of the genus *Pseudoalteromonas* are highly virulent to several economically important marine species[23] and can therefore affect different components of marine ecosystems. The genus *Vibrio* contains many species that are pathogenic for several animal species and especially oysters.[24] The *Vibrio* genus includes one of the most dangerous pathogenic bacteria known to humans, *Vibrio cholerae*, against which few antibiotic treatments are effective.[25] In a preliminary work,[7] it was first observed that IMn could inhibit the growth of *Vibrio anguillarum*, whereas EMn had no effect. This experiment consisted of applying a single deposit of 100 μg of pigment per disk. The present study confirms that marennine is active against another *Vibrio* strain and that this effect extends to lower concentrations, demonstrating that both IMn and EMn can inhibit the growth of *V. aesturianus*, which is especially encouraging for its potential use in aquaculture. In recent years, *V. aesturianus* has been implicated in high seasonal mortality of the oyster *Crassostrea gigas* in France[24] and also in other countries, such as Japan, Korea, the United States, and Australia. For a reason not yet explained, we found that the EMn form of marennine was as active as IMn. EMn accumulates in the seawater during algal growth and the culture cycle and is probably the main agent of the greening of oysters. Marennine produced by *H. ostrearia* and released into oyster ponds during the greening phenomenon could therefore

act as a natural antibiotic or bacteriostatic compound for use in oysters, counteracting the effect of pathogenic bacteria.

Oysters are filter feeders, and their gills filter huge amounts of seawater to extract and then select food particles. As a consequence, they accumulate viruses or bacteria at concentrations that can be a 1000 times higher than those in the surrounding water.[26] Some of these pathogens can be responsible for diseases or food poisoning after human consumption of shellfish containing the enteric and fecal bacteria *Enterococcus faecalis* or *Escherichia coli*. It was previously observed[7] that only IMn was active and that it inhibited the growth of *S. aureus* but not *E. coli*. In our study, no such activity was detected for either form of marennine or any of the strains tested. To rule out possible interactions between the bacterial culture medium and marennine, two bacteria, *E. faecalis* and *P. mirabilis*, were grown on normal Mueller Hinton solid medium and blood agar and the experiments were repeated; however, still no activity was detected. It therefore appears that marine and "terrestrial" forms of pathogenic bacteria differ from each other with regard to a wide range of morphological, chemical, and physiological features, including the higher sensitivity of marine bacteria to the antibacterial activity of marennine.

Antiviral Activities. The present study is the first demonstration that both the IMn and EMn forms of purified marennine exhibit a noticeable level of antiviral activity, regarding its naturally occurring substance's status. Many plants[27] and a wide diversity of marine organisms, including macro- and microalgae, produce various compounds displaying antiviral activities.[28] In algae, the active compounds are usually polysaccharides, some of which are highly sulfated.[29] The antiviral activity of highly sulfated polysaccharides was previously hypothesized using *H. ostrearia* aqueous extracts.[13] With regard to their cytotoxicity, IMn displayed a CC_{50} that was about one-half that of EMn, but this should not prevent the human consumption of "green oysters", even though EMn is expected to be the main form of marennine found on the oyster gills and, therefore, the one most likely to be consumed by oyster consumers. Although the chemical nature of marennine has not yet been elucidated, it can be hypothesized that the difference in CC_{50} and cytotoxicity between the two forms of marennine could be related to structural differences, as already evidenced.[9]

Antiproliferative Activities. Bioactive compounds extracted from algae have received increasing attention, in particular for their antiproliferative potential. For example, pigments from brown algae have displayed various activities,[30] and some algal polyphenols have been found to have antiproliferative effects against cancer cells from organs of the human digestive system.[31] Some macroalgae are edible and indeed eaten on a daily basis in some Asian countries, and therefore, they could be considered to be functional foods. Some microalgal extracts are also known to have potential antiproliferative properties.[32] In *H. ostrearia*, both forms of marennine are active against various lineages of human cancer cells. In particular, the main activity observed on the bronchopulmonary lineage NSCLC-N6 confirms a previous observation using *H. ostrearia* aqueous crude extracts.[17] This could explain why the IC_{50} observed with purified pigment was about half that found for crude extracts.[17] Indeed, whether the IMn or EMn form is considered, when expressed in specific activity, the IC_{50} values observed are in the μmol/L range, which is comparable to or lower than the IC_{50} found for various polyphenol compounds against melanoma or colon cancer cells.[33,34]

Marennine is a water-soluble pigment, possibly a polyphenol with a medium molecular weight.[9] Its mechanism of action may therefore differ from those of other polyphenols commonly described in pharmacology, most of which are small amphiphilic molecules. These small molecules can pass through the cellular membranes to reach their target and, for example, induce apoptosis.[35] Polyphenols are also responsible for a tanning effect and astringency,[36] leading to the denaturation and precipitation of proteins and, as a consequence, to the lower digestibility of some plant products, possibly acting as a defense against grazing organims.[37] Marennine activity does not seem to be specific with regard to cell lineages. The morphological changes observed in cell cultures in the presence of marennine, in combination with its antiproliferative activities, could be the consequence of a tanning effect on proteins contained in the cell membranes. This tanning effect results in changes in the proteins on the cell surface and changes in the cellular matrix and the ability of the cell to adhere to the substrate or to each other. This hypothesis is supported by the slight inhibition and occasional stimulation of growth observed in cells from the non-adherent lineage. These observations are consistent with possible activation of apoptotic pathways[12] via interaction with the EMn matrix.

Despite the fact that they have been consumed for centuries, marennine-colored oysters have not been associated with any specific human disorder. Moreover, the results presented here allow us to hypothesize that, in addition to its antioxidant properties,[11] marennine and green oysters could be viewed as a "healthy" food. Indeed, it has been shown that large polyphenol molecules, which display antibacterial activities, are commonly metabolized before being absorbed, resulting in smaller molecules that may have different properties, although they usually also display antibacterial activity.[38] At present, it is impossible to surmise what degradation products of marennine are produced after digestion, because its structure is still unknown. Moreover, it could be difficult to investigate these products because marennine seems to be resistant to heat, light, and acidic or enzymatic degradation.[9] Nevertheless, as an increasing number of microalgae are exploited to produce bioactive substances,[39] the present results are encouraging enough to pursue further studies on possible uses of marennine in aquaculture or for the food industry.

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Notes

The authors declare no competing financial interest.

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