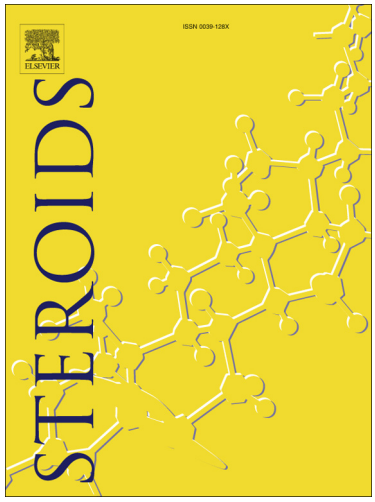


Antifouling activity of peracetylated cholic acid, a natural bile acid derivative

Miriam Pérez, Marianela Sánchez, Mónica García, Laura P. Patiño C, Guillermo Blustein, Jorge A. Palermo

PII: S0039-128X(19)30098-4
DOI: https://doi.org/10.1016/j.steroids.2019.05.006
Reference: STE 8414

To appear in: *Steroids*

Received Date: 16 March 2019
Revised Date: 30 April 2019
Accepted Date: 15 May 2019

Please cite this article as: Pérez, M., Sánchez, M., García, M., Patiño C, L.P., Blustein, G., Palermo, J.A., Antifouling activity of peracetylated cholic acid, a natural bile acid derivative, *Steroids* (2019), doi: https://doi.org/10.1016/j.steroids.2019.05.006

Antifouling activity of peracetylated cholic acid, a natural bile acid derivative

Miriam Pérez[1,2], Marianela Sánchez[3,4,a], Mónica García[1], Laura P. Patiño C.[3,4,b], Guillermo Blustein[1,5], and Jorge A. Palermo*[,3,4]

[1] Centro de Investigación y Desarrollo en Tecnología de Pinturas- CIDEPINT, Calle 52 e/ 121 y 122- La Plata B1900AYB, Argentina.

[2] Universidad Nacional de La Plata, Facultad de Ciencias Naturales y Museo, Calle 60 y 122, 1900 La Plata, Argentina.

[3] Universidad de Buenos Aires, Departamento de Química Orgánica - Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, Pabellón 2 - (1428) - Buenos Aires - Argentina.

[4] CONICET-Universidad de Buenos Aires, Unidad de Microanálisis y Métodos Físicos en Química Orgánica (UMYMFOR), Buenos Aires, Argentina.

[5] Universidad Nacional de La Plata, Facultad de Ciencias Agrarias y Forestales, Calle 60 y 119, 1900 La Plata, Argentina.

*Corresponding author: palermo@qo.fcen.uba.ar Phone: 5411-4576-3385 ORCID: 0000-0002-0892-9389

[a] Present Address: Centro de Investigaciones y Transferencia de Villa María (CITVM-CONICET), Universidad Nacional de Villa María, Campus Universitario, Arturo Jauretche 1555, Villa María, Córdoba, Argentina.

[b] Present Address: Centro de Investigación de Productos Naturales y Biotecnología (CIPNABIOT), Universidad Autónoma de Chiriquí, El Cabrero, David, Chiriquí, Panamá.

Abstract

The antifouling activity of peracetylated cholic acid (**1**), a bile acid derivative which was isolated in a previous work as a natural product from the Patagonian sponge *Siphonochalina fortis*, was evaluated in laboratory and field trials. Toxicity and settlement assays were performed with the mussel *Mytilus edulis platensis*, while the field trials were carried out by addition of the compound to experimental soluble-matrix paints, which were then tested in the sea. The results obtained in this work show that **1** has a good antifouling activity and low toxicity, and the paints aditivated with 0,6 % Wt showed promisory performances in the field trials at the sea. These results confirm the previous hypothesis that the few acetylated and lipophilic bile acid derivatives isolated from marine invertebrates may act as natural antifoulants. Compound **1** is a natural, biodegradable product that can be easily prepared from cholic acid, which in turn can be isolated in industrial scale from cattle bile. All these facts make cholic acid a good scaffold for the preparation of derivatives, which can be natural product-like, effective and sustainable antifouling additives for marine paints and other applications.

Keywords

Antifouling activity – Acetylated bile acids – Marine Invertebrates - *Siphonochalina fortis*

1- Introduction

Marine biofouling is defined as the settlement and growth of a community of organisms on the surface of objects, either natural or artificial, which are submerged in the sea [1], and is facilitated by biological, physical, chemical surface-associated cues [2-3]. In the case of benthic, filter feeding marine invertebrates such as sponges or cnidarians, biofouling reduces the access to nutrients and sunlight, and, at the same time, the added weight of fouling organisms increases the risk of dislodgement from their substrates. Since in the marine environment the control of epibionts is a key feature for survival, it is no surprise that many of the more than 22000 marine natural products that have been reported up to date play significant roles as antifouling agents [4-6]. Marine biofouling is also an important issue for the shipping industry and for all other human activities that take place in the sea or involve the use of seawater, such as offshore platforms, underwater pipelines, desalination plants and mariculture facilities [7-9]. For example, it is estimated that ships with fouled hulls, consume up to 40 % more fuel [10]. Biofouling also produces pipe-clogging in underwater facilities, and this leads to a heavy increase of maintenance costs. There are also ecological concerns about the effect of biofouling on ship hulls, since invading species can then be transported via maritime trade. All these problems have led to intense research on antifouling technologies, and, among the different alternatives, the use of antifouling coatings is still the preferred method for the control of biofouling. Tipically, this methodology is based on a paint system consisting of a primer/base coat and a compatible copper-based antifouling coating [11]. Traditionally, successful antifouling technologies incorporated either metallic or organic biocides to inhibit larval settlement. However, the deleterious effects that some of these biocides cause to marine ecosystems have boosted the research efforts towards the development of environmentally-friendly antifouling materials [12].

In a previous publication, several acetylated bile acids (**Fig. 1**) were isolated from the Patagonian sponge *Siphonochalina fortis*. [13]. This finding was a surprise since the corresponding free bile acids were not detected in the sponge extract. Also, in accordance to their typical biological roles, bile acids are not among the usual classes of steroids found in marine invertebrates. These facts, together with the observation that the surface of the sponge was free from fouling organisms, suggested that these acetylated bile acids could have a possible role as antifoulants.

Bile acids, which are the end products of cholesterol catabolism in mammals, play important roles in human metabolism, especially in the absorption of lipids in the small intestine and in the regulation of cholesterol in bile. However, bile acids and alcohols are not tipically biosynthesized by marine invertebrates, and these compounds are replaced by other polar substances that can act as emulsifiers, such as fatty acylsarcosyltaurines and peptides. In the most simple invertebrates, such as sponges, there are no digestive juices and hence no metabolic need for these substances. Among the plethora of structurally diverse steroids isolated from marine invertebrates [14, 15], there are only a few reports of bile acids and derivatives, and these isolations have been reported mainly from octocorals [16-18], and sponges [19]. These infrequent findings of bile acid derivatives in marine invertebrates are usually considered to be of symbiotic microbial origin, since there are reports of several taxa of marine bacteria associated with sponges, which are capable of producing bile acids [20]. An interesting structural feature of these marine bile acid derivatives is that they usually have increased lipophilicity, for example by acetylation of their hydroxyl groups. Since the acetylation of polar compounds reduces their water solubility, it is possible that this structural feature may be a requirement for an ecological action. This is especially desirable in the case of interactions that take place at the surface of the invertebrate, such as an antifouling activity. Reduced water solubility allows a high concentration of the bioactive compound on the surface of the organism and, at the same time, lowers the diffusion of the substance along the water column.

In this work, we provide experimental evidence of the antifouling activity of peracetylated cholic acid, one of the compounds originally isolated from *S. fortis*, by means of laboratory and field experiments. Peracetylated cholic acid (**1**) was prepared by a standard acetylation procedure, and tested for antifouling activity in laboratory and field trials. Toxicity and settlement assays were performed with the mussel *Mytilus edulis platensis*, while the field trials were carried out by addition of the compound to experimental soluble-matrix paints, which were then tested in the sea. The results of this work may explain the presence and ecological role of bile acid derivatives in marine invertebrates, and provide a foundation for the development of new environmentally-friendly antifouling derivatives based on bile acids.

2- Experimental

2.1 Fouling organisms

Mussels (*Mytilus edulis platensis*) were collected together with their rock substratum from intertidal rocks at Playa Chica, Mar del Plata, Argentina (38º08' 17''S, 57º 31' 18''W). In the laboratory, individuals with a valve length between 5-8 mm were then disaggregated. The shells were washed and brushed in order to eliminate adhered organic or inorganic deposits, and all the mussels were conditioned in artificial seawater (ASTM D1141/75) with the following physicochemical properties: pH 8.2-8.3, salinity 33-35‰, temperature 22±2ºC, with suitable aeration and natural light. The mussels were not fed during the experimental period.

2.2 Acetylation of Cholic acid

Cholic acid (100 mg) was dissolved in a vial in 0.5 mL of dry pyridine and 0.5 mL of acetic anhydride, and left overnight at room temperature with stirring. The crude mixture was poured over 25 g of crushed ice, which was previously acidified with conc. HCl. After the ice melted, the mixture was diluted with 50 mL of water and extracted three times with 50 mL of methylene chloride (CH_2Cl_2). The organic layer was washed with water, and then taken to dryness at reduced pressure, to give a quantitative yield of pure peracetylated cholic acid (**1**). The NMR spectra of the synthetic compound were identical to those of the natural product [13].

2.3 Preparation of test solutions

A stock solution was prepared for the experiments by dissolving peracetylated cholic acid (**1**) in dimethylsulfoxide (DMSO). Then, eight dilutions in artificial seawater were arranged to obtain the following final concentrations: 0.102 µM; 1.02 µM; 10.2 µM; 102 µM; 136.2 µM; 204.3 µM; 271.6 µM; 340 µM. Seawater + DMSO was used as control. Experiments were performed by triplicate.

2.4 Toxicity assays

Toxicity and settlement assays were carried out following the methodology (modified) of Wilsanand et al. [21]. These assays were performed in 24-well Iwaki plates containing 2 mL of test or control solution and one mussel per well. The mussels were added in a minimal volume of seawater. Tests plates were prepared in the dark and incubated at 22±2ºC [22]. The mussels were inspected after an incubation of 72 h, and individuals were considered as "inactive" or "dead" when they were unsettled and/or had their valves open. Inactive or dead individuals were counted, and the concentration necessary to inactivate or kill 50% of the

mussel population (LC_{50}) was calculated. Finally, mussels were washed and transferred to clean seawater for 24 h to detect a temporary or permanent effect (recovery test).

2.5 Settlement assays

Incubation and observation were carried out as previously mentioned. In particular, settled and unsettled individuals were registered after 24 h of exposure to the test solutions, and the effective concentration to avoid 50% settlement (EC_{50}) was determined.

Additionally, the therapeutic ratio (TR) of compound **1** was also calculated. TR is defined as LC_{50}/EC_{50} and indicates whether settlement inhibition is due to the toxicity of the compounds or related to other mechanisms [23]. A comparison of LC_{50} and EC_{50} values provides insight to the possible working mechanisms of the tested compounds. However, a thorough understanding of compound toxicity is given by recovery tests, which determine if the effect produced by a tested compound is reversible or permanent.

2.6 *Paint preparation*

For the preparation of soluble matrix antifouling paints, colophony (WW rosin) was used as binder and oleic acid as plasticizer. The paint was prepared in 1.0 L jars of a ball mill at adequate operating conditions to achieve an efficient dispersion. Briefly, antifouling paints were prepared by dissolution of colophony and oleic acid in a xylene/white spirit/methyl isobutyl ketone mixture (1:1:0.5) using a high-speed disperser; the ball mill was then loaded with this mixture ("vehicle"), zinc oxide and calcium carbonate ("pigments"), and dispersed for 24 h. After that, the paint was filtered through a Lycra™ grid in order to eliminate environmental dust and/or bigger particles, and fractionated in two portions, one of which was used as a negative control and the other as treatment. A control paint was formulated with the following composition (as weight %): colophony (22), oleic acid (4.6), zinc oxide (34.6), calcium carbonate (11.1) and solvent (25.4). For treatment, compound **1** was dissolved in 1 mL of MeOH, and then incorporated into the matrix paints at 0.6% Wt. Finally, paints were dispersed during 1h.

2.7 *Field trials*

Antifouling paints were applied on acrylic tiles (4×12 cm^2), which were previously sandblasted and degreased with toluene. Four layers of paint were applied leaving a drying time of 24 h between each coat to obtain a final dry thickness of 100±5 μm. Series of panels were arranged in aluminum racks and submerged

in a marina at Mar del Plata harbor (Argentina). Controls consisted of uncoated plates, and plates coated only with paint matrix. Field experiments were evaluated after exposure times of 45 and 90 days in the sea during the summer season (December- March) which is a period with intense settlement of organisms. Cover percentages were estimated by the dot grid method that consists in the identification and frequency of settled organisms located at 25 random points. The methodology is carried out by exhaustive taxonomical identification under stereomicroscope (Leica Stereozoom S8 APO equipped with a DFC 295digital camera and analytical software) and optical microscope. All tests were performed in triplicate [24].

2.8 *Statistical analysis*

All statistical analyses were performed with IBMSPSS 22. The normality assumption was verified with the Shapiro-Wilk's test [25]. The differences between treatment and controls were determined by one-way analysis of variance (ANOVA) and differences were considered to be significant at $p < 0.05$. Calculations of LC_{50} and EC_{50} with 95% confidence intervals were done with Probit analysis [26].

3 Results

Laboratory tests were performed with the mussel *M. edulis platensis*, a species commonly found in the fouling community at Mar del Plata harbor (Argentina), the location where the field trials were also performed. Adult mussels are convenient test organisms based on their biological abilities, which include their capacity to sense chemical and physical characteristics of a substratum using the foot, the ability to secret byssal threads for attachment to a surface, to release byssal threads from unfavourable surfaces, as well as the ability to reattach to a surface by secretion of new byssal threads [27]. In addition, mussels can be used as bioindicators to study the general antifouling potency of a biocide against macroorganisms.The results of the toxicity and refreshing tests are shown in **Fig**. **2**.

Significant differences between treatments and control were observed ($p<0.05$). These results indicate that the percentage of mussel inactivity or mortality increases significantly at concentrations of compound **1** higher than 10.21 μM. In this case, the LC_{50} was determined at 151.12 μM. On the other hand, the percentage of mussel recovery after transfer to fresh artificial seawater was estimated as 75% of mussel

population in a concentration range of 0-136.2 μM. At higher concentrations of compound **1** the recovery percentage was lower.

The settlement assays of *M. edulis platensis* on wells showed significant differences at concentrations of compound **1** higher than 18.03 μM. The effective concentration to inhibit 50% of mussel population was established as 118.18 μM (**Fig. 3**). Additionally, the TR for the tested compound was established as 1.3.

Based on the previous results, compound **1** was then tested in the sea, by addition to experimental paints. Soluble matrix paints are one of the main technologies used in antifouling coatings. In these paints, the matrix is formed mainly by colophony resin, which, at the slightly basic pH of seawater is slowly dissolved, releasing at the same time a constant flow of the antifoulant to the tested surface. Test paints were prepared with the addition of peracetylated cholic acid (**1**). Test acrylic tiles were coated with these paints and then submersed in the sea for several weeks. This test can provide a realistic picture of the antifouling activity of a given compound. In these assays, the painted surface is presented to a wide array of species and *phyla* of the benthic community under natural conditions of water flow and temperature, while being exposed to a natural supply of larvae and algal spores. The paints containing compound **1** showed promising antifouling potencies after 45 and 90-day exposure of the painted panels in the sea (**Fig. 4, 5 and 6**).

The settlements of the anemona *Anthotoe chilensis*, the sand-tube builder *Polydora* sp. and the solitary ascidian *Ciona intestinalis* were completely inhibited in all the experiments. Moreover, the additivated paint reduced significantly the settlement of the calcareous tubeworm *Hydroides elegans*, the bryozoan *Bugula* sp. and the green algae *Enteromorpha intestinalis.* The settlement of the colonial ascidian *Botryllus* sp. and the sand-tube builder *Corophium* sp. were significantly reduced during the first 45 days but were not affected after 90 days. In contrast, the adhesion of brown alga *Ectocarpus* sp. was not affected by the paint and thus colonized the treated panels. It is important to remark that settlers like the alga *Ceramium* sp. and sponges, which arrive at later stages to the fouling community, were also inhibited by peracetylated cholic acid antifouling paint.

4. Discussion

Marine antifouling compounds belong to diverse chemical classes and display a great structural variety. Most of them are non-polar substances with low water solubility, and among these antifouling marine natural products there are a number of steroid derivatives. However, as previously stated, bile acid derivatives are a rarity among marine natural products, and there are no previous reports on antifouling activity for this class of steroids. Besides its established roles in the metabolism of lipids, some additional biological and technological activities have been described for cholic acid derivatives [28-30]. Taking into account that antifouling activity can be related to antibiotic activity, of special interest are also a series of cholic acid derivatives with antibiotic activity [31]. In particular, the group of Savage has developed a series of cholic acid derivatives, with different acyl groups at the three hydroxylated positions and additional alkyl chains esterifying the acid in order to increase lipophilicity. These compounds have intense antibiotic activity against a wide array of bacteria, including Gram-negative, and biofilm-forming bacteria [32-37]. Natural acetylated bile acids may have a similar antibiotic effect on the bacteria that form the primary film, one of the first steps in the development of a fouling community [8]. However, since compound **1** also showed some toxicity against mussels in the laboratory tests, there is the possibility that several ways of action may act simultaneously to produce the observed antifouling effect. It must be stated that the peracetylated bile acids originally isolated from *S. fortis* did not show significant genotoxic activity at the concentrations found in the sponge [13], and thus were not responsible for the genotoxic activity originally detected in the sponge extract [38]. However, the results of the present work clearly demonstrate the antifouling action of the acetylated bile acids, which are the main secondary metabolites in the extract of the sponge *S. fortis*.

The results obtained with the antifouling paints open the way for the development of biotechnological applications of lipophilic derivatives of bile acids. A logical step in the search of natural antifouling agents was to look at the marine environment. However, marine natural products generally face a serious sustainability issue that prevents their technological use. The main problem with the use of these natural products is the amount of compound that may be necessary for an industrial use as antifouling agents in paints compared to the yields obtained from their natural sources. In many cases, these secondary metabolites are biosynthesized only in small amounts by marine invertebrates or by their symbiotic microorganisms. This would require large-scale collections of invertebrates to obtain the necessary amounts, which will undoubtedly produce disastrous ecological consequences. Another issue is the seasonal and

geographical variability in the production of secondary metabolites, which is related to the composition of the community of symbiotic microorganisms. Mariculture of marine invertebrates (which is a laborious process, especially with delicate or slow-growing invertebrates) or biotechnological production from symbiotic strains look like suitable alternatives. However, there is no guarantee that an invertebrate cultured outside its original environment, or a symbiont grown outside its biological host will produce the required secondary metabolites, compounds which were biologically designed as a response to the characteristics of a particular ecosystem. For these reasons, nowadays, marine invertebrates are considered more as a source of inspiration for the development of natural product-like antifouling compounds than a sustainable source of natural antifoulants [39].

In the case of acylated bile acid derivatives, this sustainability issue is overcome. Some bile acids, especially cholic acid, can be purified in large quantities at a very low cost from cattle bile obtained from slaughterhouses. This makes bile acids an accessible and sustainable natural resource, especially in cattle producing countries. Acylation of bile acids is a simple and high-yielding procedure. All these facts point towards a possible large-scale production of acylated bile acids for industrial use as antifouling additives in marine paints.

5. Conclusions

In this work, experimental evidence is provided on the antifouling activity of peracetylated cholic acid, a compound that was unexpectedly isolated from a marine sponge, and that can be easily prepared in large quantities from the readily available cholic acid. The antifouling activity of peracetylated cholic acid was first tested in the laboratory against the mussel *M. edulis platensis* and then by incorporation to experimental soluble-matrix paints which were tested in the ocean. The biosynthetic origin of the compound originally isolated from the sponge was probably a bacterial symbiont, and acetylation by the marine invertebrate or by the symbiont itself at a later stage, produced the highly lipophilic derivatives that provide antifouling chemical defense for the sponge. This is a new biological activity for bile acid derivatives, and these results reinforce the idea that the other previous examples of lipophilic derivatives of bile acids isolated from marine invertebrates may have similar antifouling activities as well. The tests of compound **1** in the ocean with experimental paints showed a potent antifouling activity against a wide variety of species belonging to

different *phyla*. Traditionally, in order to be selected as a promising new antifouling additive, compounds needed to have an LC_{50}/EC_{50} (TR) relationship higher than 1, although recent trends consider that in non-toxic antifoulants the LC_{50}/EC_{50} should be larger [40]. However, the possibility of a reversible effect (as in the case of a narcotic effect) should always be taken into account, in order to determine the toxicity degree of a compound. In the case of compound **1**, although the TR for the tested compound was 1.3, the non-toxic effect was reversible, as confirmed by the recovery tests. The fact that compound **1** is a natural product, and that has been isolated previously from marine invertebrates must also be taken into account. Although the toxicity/activity ratio of compound **1** in the laboratory tests is still not large enough for an industrial use in antifouling coatings, it can probably be optimized by structural diversification. Cholic acid then emerges as an interesting scaffold for the preparation of more active and/or less toxic derivatives that may be suitable for use as antifouling additives in paints, especially since acylation of bile acids is a simple chemical procedure. Bile acids, and especially cholic acid, can be purified in large quantities at a very low cost from cattle bile. All these facts make bile acids an accessible and sustainable natural resource, especially in cattle-producing countries, that can be used as starting material for the preparation of natural product-like antifoulants in an industrial scale.

Acknowledgements

Research at the University of Buenos Aires was supported by grants from CONICET (PIP 2014-2016 Nº 11220130100523CO), UBA (UBACyT 2014-2017 Nº 20020130100457BA) and ANPCyT (PICT 2014-2063). M.S., G.B and J.A.P are researchers of CONICET. L.P.C. thanks SENACYT (Panamá) for a doctoral fellowship.

References

[1] J. Lewis, Marine biofouling and its prevention on underwater surfaces, Mater. Forum 22 (1998), 41–61.

[2] D. Rittschof, N. Sasikumar, D. Murlless, A. Clare, C. Gerhart, J. Bonaventura, Recent developments in biofouling control, Oxford and IBH Publ. Co., New Delhi, India, 1994.

[3] A. Clare, N. Aldred, Surface colonisation by marine organisms and its impact on antifouling research, in: C. Hellio, D. Yebra (Eds.), Advances in marine antifouling coatings and technologies, Cambridge: Woodhead Publishing, UK, 2009, pp. 46-79.

[4] N. Fusetani, Antifouling marine natural products, Nat. Prod. Rep. 28 (2011), 400-410.

[5] P. Krug, Defense of benthic invertebrates against surface colonization by larvae: A chemical arms race. Marine Molecular Biotechnology, Springer, Berlin, 2006.

[6] P.Y.Qian, S.Y. Xu, Antifouling activity of marine natural products, in: E. Fattorusso, .H. Gerwick, O. Taglialatela-Scafati (Eds.), Handbook of marine natural products, Springer Science, 2012, pp. 749-821.

[7] L. Evans, N. Clarkson, Antifouling strategies in the marine environment, J. Appl. Bacteriol.74 (2012), 119S-124S.

[8] H. Dahms, S. Dobretsov, P. Qian , The effect of bacterial and diatom biofilms on the settlement of the bryozoan *Bugula neritina*. J. Exp. Mar. Biol. Ecol. 313 (2004), 191-209.

[9] D. Inbakandan, C. Kumar, L. Stanley Abraham, R. Kirubagaran, R. Venkatesan, S. Ajmal Khan, Silver nanoparticles with anti microfouling effect: a study against marine biofilm forming bacteria, Colloids Surf. B. 111 (2013), 636-643.

[10] D. Yebra, S. Kiil, K. Dam-Johansen, Antifouling technology-past, present and future steps towards efficient and environmentally friendly antifouling coatings, Prog. Org. Coat. 50 (2004), 75–104.

[11] P. Earley, B. Swope, K. Barbeau, R. Bundy, J. McDonald, I. Rivera-Duarte, Life cycle contributions of copper from vessel painting and maintenance activities, Biofouling 30 (2014), 51–68.

[12] J. Callow, M. Callow, Trends in the development of environmentally friendly fouling-resistant marine coatings, Nat Commun. 2 (2011), 1–10.

[13] L. Patiño Cano, S. Bartolotta, N. Casanova, G. Siless, E. Portmann, L. Schejter, J. Palermo, M. Carballo, Isolation of acetylated bile acids from the sponge *Siphonochalina fortis* and DNA damage evaluation by the comet assay, Steroids 78 (2013), 982-986.

[14] R.G. Kerr, B.J. Baker BJ, Marine sterols, Nat. Prod. Rep. 8 (1991), 465-497.

[15] J.W. Blunt, A. Carroll, B. Copp B, R. Davis, R. Keyzers, M. Prinsep, Marine natural products, Nat. Prod. Rep. 35 (2018), 8-53.

[16] S.C. Lievens, H. Hope, T.F. Molinski, New 3-oxo-chol-4-en-24-oic acids from the marine soft coral *Eleutherobia* sp. J. Nat. Prod. 67 (2004), 2130-2132.

[17] P.J. Sung, L.S. Fang, Y.P. Chen, W.C. Chen, W.P. Hu, C.L. Ho, S.C. Yu, 5β-Steroids from the gorgonian coral *Junceella fragilis* (Ellisellidae), Biochem. Syst. Ecol. 34 (2006), 64-70

[18] W.C. Chen, J.H. Sheu, L.S. Fang, W.P. Hu, P.J. Sung, 3α,7α,12α-Triacetoxy-5β-cholanic acid, a steroid from the Formosan soft coral *Alcyonium sp.* (alcyoniidae), Nat. Prod. Res. 20 (2006), 748-753.

[19] Z. Lu, R.M. Van Wagoner, M.K. Harper, J.N. Hooper, C.M. Ireland, Two ring-A-aromatized bile acids from the marine sponge *Sollasella moretonensis*, Nat. Prod. Commun. 5 (2010), 1571-1574.

[20] S. Maneerat, T. Nitoda, H. Kanzaki, F. Kawai, Bile acids are new products of a marine bacterium, *Myroides* sp. Strain SM1, Appl. Microbiol. Biotechnol. 67 (2005), 679-683.

[21] V. Wilsanand, A. Wagh, M. Bapuji M, Antifouling activities of marine sedentary invertebrates on some macrofoulers, Indian J. Mar. Sci. 28 (1999), 280-284.

[22] A. Davis A, C. Moreno C, Selection of substrata by juvenile *Choromytilus chorus* (Mytilidae): are chemical cues important?, J. Exp. Mar. Biol. Ecol. 191 (1995), 167-180.

[23] A. Clare, D. Rittschof, D.J. Gerhart, I.R. Hooper, J. Bonaventura, Antisettlement and narcotic action of analogues of diterpene marine natural product antifoulants from octocorals, Mar. Biotechnol. 1 (1999), 427–436.

[24] M. Foster, C. Harrold, D. Hardin D, Points versus photo quadrat estimates of the cover of sessile marine organisms, J. Exp. Mar. Biol. Ecol. 146 (1991), 193-203.

[25] S. Shapiro, M. Wilk, An analysis of variance test for normality (complete samples), Biometrika 52 (1965), 591-611.

[26] D. Finney D, Probit Analysis, third ed., Cambridge University Press, Cambridge, UK (1971).

[27] C. Ayala, M. Clarke, C. Riquelme, Inhibition of byssal formation in *Semimytilus algosus* (Gould, 1850) by a film-forming bacterium isolated from biofouled substrata in northern Chile, Biofouling 22 (2006), 61-68.

[28] A. Enhsen, W. Kramer, G. Wess, Bile acids in drug discovery, DDT 3 (1998), 409-418.

[29] E. Virtanen, E. Kolehmainen , Use of bile acids in pharmacological and supramolecular applications, Eur. J. Org. Chem. (2004), 3385-3399.

[30] M. Blanchet, J.M. Brunel, Bile acid derivatives: From old molecules to a new potent therapeutic use: An overview, Curr. Med. Chem., 25 (2018), 3613-3636.

[31] H. Willemen, L. Smet, A. Koudijis, M. Stuart, I. Heikamp-de Jong, G. Marcelis, T. Antonius, E. Sudholter, Micelle formation and antimicrobial activity of cholic acid derivatives with three permanent ionic head groups, Angrew Chem Int Ed 41 (2002), 4275-4277.

[32] C. Li, A. Peters, E. Meredith, G. Allman, P. Savage, Design and synthesis of potent sensitizers of Gram-negative bacteria based on a cholic acid scaffolding, J Am Chem Soc 120 (1998), 2961-2962.

[33] C. Li, L. Budge, C. Driscoll, B. Willardson, G. Allman, P. Savage, Incremental conversion of outer-membrane permeabilizers into potent antibiotics for Gram-negative bacteria, J Am Chem Soc 121(1999), 931-940.

[34] E. Schmidt, J. Boswell, J. Walsh, M. Schellenberg, T. Winter, C. Li, G. Allman, P. Savage, Activities of cholic-derived antimicrobial agents against multidrug-resistant bacteria, J Antimicrob Chemoth 47 (2001), 671-674.

[35] M.M. Hashemi, B.S. Holden, B. Durnas, R. Bucki, P. Savage, Ceragenins as mimics of endogenous antimicrobial peptides, J Antimicrob Agents 3 (2017), 141.

[36] M.M. Hashemi, J. Rovig, S. Weber, B. Hilton, M.M. Forouzan, P,B. Savage PB, Susceptibility of colistin-resistant, Gram-negative bacteria to antimicrobial peptides and ceragenins Antimicrob Agents Ch 61 (2018), e00292-17.

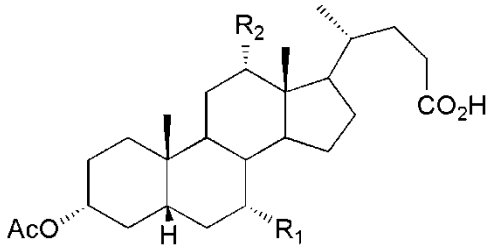
[37] M.A. Olekson, T. You, P.B. Savage, K.P. Leung, Antimicrobial ceragenins inhibit biofilms and affect mammalian cell viability and migration *in vitro*, FEBS Open Bio 7 (2017), 953-967.

[38] S.A. Bartolotta, M.A. Scuteri, A.S. Hick, J.A. Palermo, M.F. Rodríguez Brasco, E. Hajdu, B. Mothes, C. Lerner, M. Campos, M.A. Carballo, Evaluation of genotoxic biomarkers in extracts of marine sponges from Argentinean South Sea, J Exp Mar Biol Ecol 369 (2009), 144-147.

[39] Y. Nogata, Y. Kitano, In: N. Fusetani, E. Clare E (Eds.), Progress in molecular and subcellular biology, 42, Springer Berlin, 2006, pp. 87-104.

[40] P.Y. Qian, Y. Xu, N. Fusetani, Natural products as antifouling compounds: recent progress and future perspectives, Biofouling 26 (2010), 223-234.

Figures



1 R_1= OAc, R_2= OAc, cholic acid, 3,7, 12-triacetate
2 R_1= OAc, R_2= OH, cholic acid, 3,7-diacetate
3 R_1= H, R_2= OAc, deoxycholic acid, 3,12-diacetate

Fig. 1 Acetylated bile acids isolated from *S. fortis*

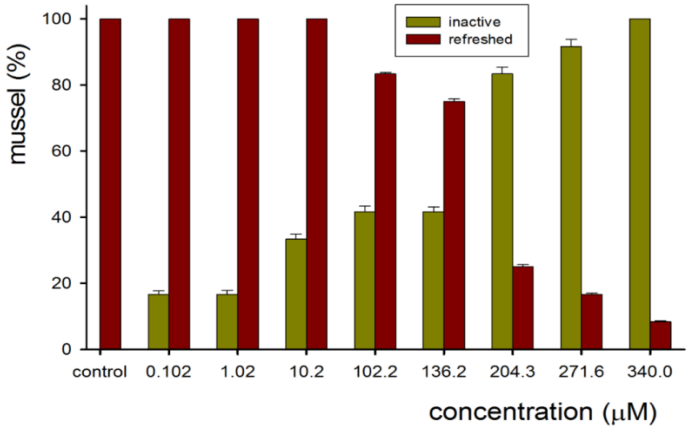


Fig. 2 Toxicity (green bars) and refreshing (brown bars) tests

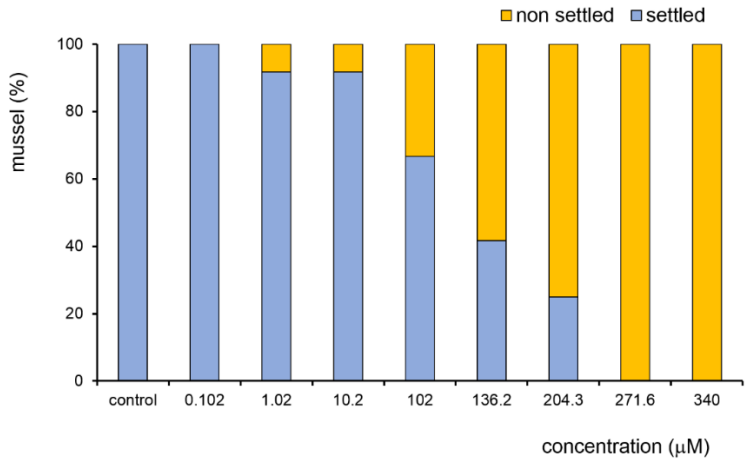


Fig. 3 Settlement test.

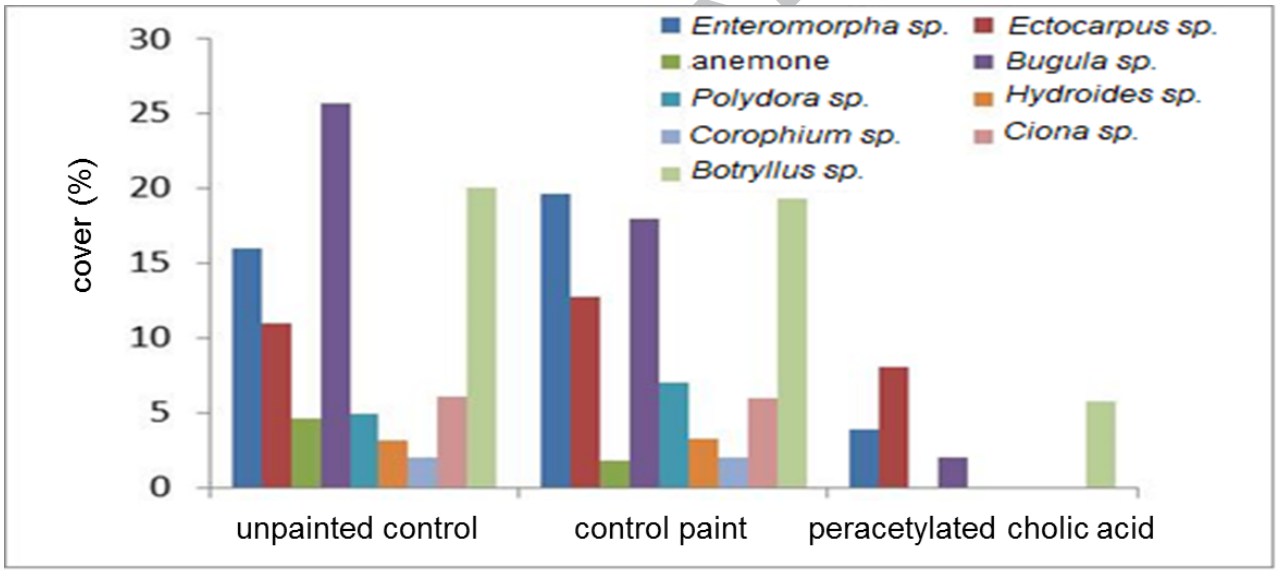


Fig. 4 Settlement on panels after 45 days exposure.

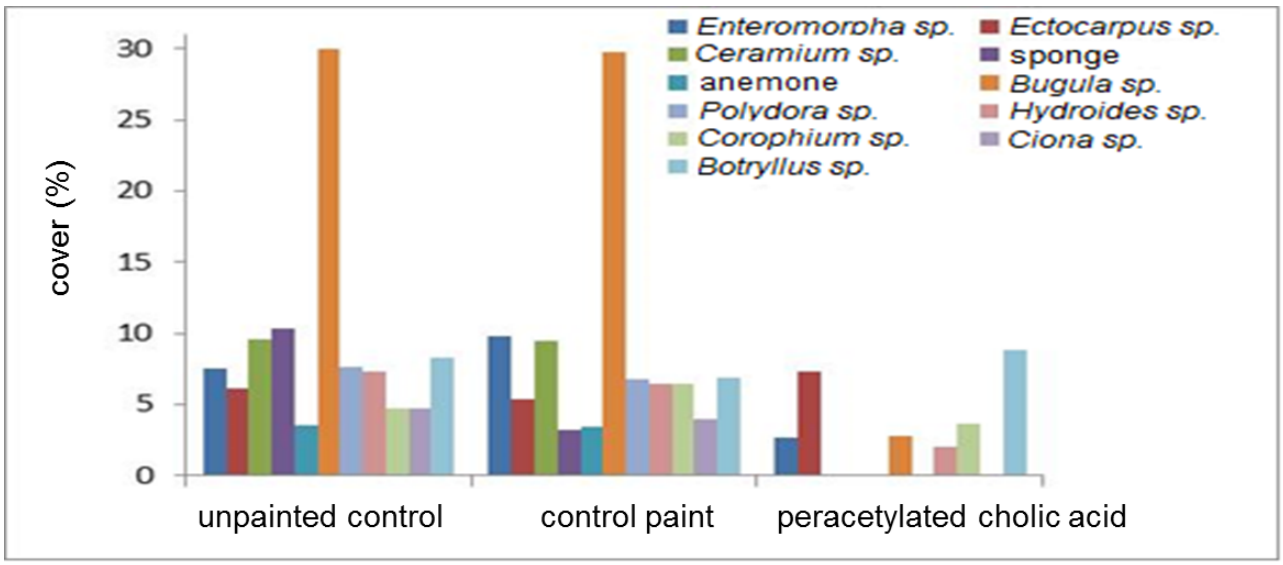


Fig. 5 Settlement on panels after 90 days exposure.

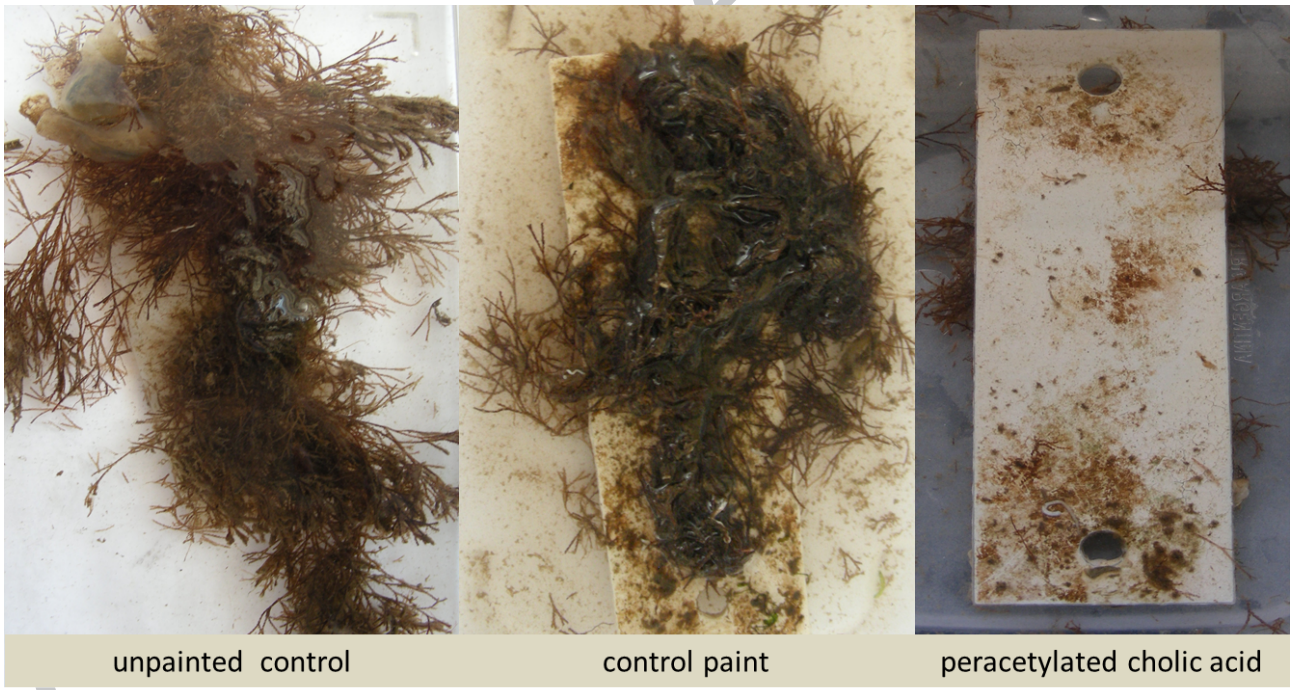


Fig. 6 Settlement on panels after 90 days exposure.

Antifouling activity of peracetylated cholic acid, a natural bile acid derivative isolated from a Patagonian sponge

Miriam Pérez, Marianela Sánchez, Mónica García, Laura P. Patiño C., Guillermo Blustein, and Jorge A. Palermo*

Credit Author Statements

Miriam Pérez: Investigation- Methodology

Marianela Sánchez: Investigation- Methodology

Mónica García: Investigation- Methodology

Laura P. Patiño C.: Investigation- Methodology

Guillermo Blustein: Supervision – Formal Analysis

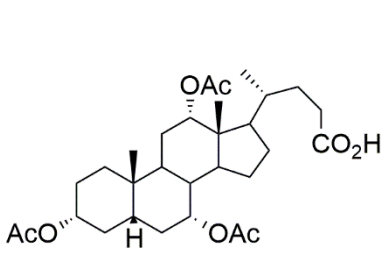
Jorge A. Palermo: Supervision – Resources –Conceptualization - Writing

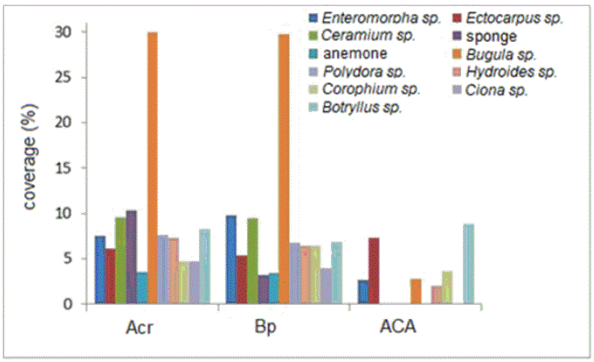
Antifouling activity of peracetylated cholic acid, a natural bile acid derivative

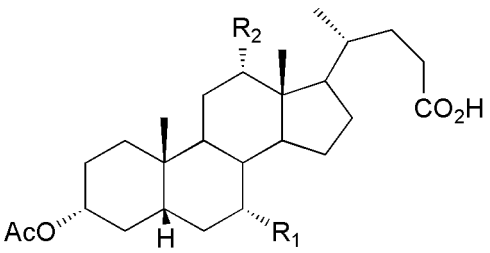
Miriam Pérez, Marianela Sánchez, Mónica García, Laura P. Patiño C., Guillermo Blustein, and Jorge A. Palermo*

Highlights

- The antifouling activity of peracetylated cholic acid was evaluated in laboratory and field trials.
- Peracetylated cholic acid had been previously isolated from a marine sponge.
- Field trials were performed by incorporation to experimental paints and then tested in the sea.
- Peracetylated cholic acid displayed very potent antifouling activity.
- Bile acids are good scaffolds for the development of environmentally friendly antifoulants.







1 R_1= OAc, R_2= OAc, cholic acid, 3,7, 12-triacetate
2 R_1= OAc, R_2= OH, cholic acid, 3,7-diacetate
3 R_1= H, R_2= OAc, deoxycholic acid, 3,12-diacetate