Isolation and Antifouling Activity of Azulene Derivatives from the Antarctic Gorgonian Acanthogorgia laxa

Article *in* Chemistry & Biodiversity · November 2017

DOI: 10.1002/cbdv.201700425

Laura P Patiño Cano
The Autonomous University of Chiriqui

Monica Garcia
Universidad Católica de Colombia

Guillermo Blustein
Centro de Investigación y Desarrollo en Tecnología de Pinturas

Ralf Cordeiro
Universidade Federal de São Paulo

CHEMISTRY & BIODIVERSITY

Isolation and Antifouling Activity of Azulene Derivatives from the Antarctic Gorgonian *Acanthogorgia laxa*

Laura P. Patiño Cano,[a,b,1] Rodrigo Quintana Manfredi,[a,b] Miriam Pérez,[c,d] Mónica García,[c] Guillermo Blustein,[c,e] Ralf Cordeiro,[f] Carlos D. Pérez,[f] Laura Schejter,[g,h] and Jorge A. Palermo*[a,b]

[a]Departamento de Química Orgánica – Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 1428 Buenos Aires, Argentina, e-mail: palermo@qo.fcen.uba.ar

[b]Unidad de Microanálisis y Métodos Físicos en Química Orgánica (UMYMFOR), CONICET-Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 1428 Buenos Aires, Argentina, e-mail: palermo@qo.fcen.uba.ar

[c]Centro de Investigación y Desarrollo en Tecnología de Pinturas-CIDEPINT, Calle52 e/121 y 122, B1900AYB La Plata, Argentina

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

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

[f]GPA-Grupo de Pesquisa em Antozoários, Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, Rua Alto do Reservatório s/n, Bela Vista CEP: 55608-680, Vitória de Santo Antão/PE, Brazil

[g]Laboratorio de Bentos, Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo 1, B7602HSA Mar del Plata, Argentina

[h]Instituto de Investigaciones Marinas y Costeras (IIMyC-CONICET), Rodriguez Peña 4046 Nivel 1, 1260 Mar del Plata, Argentina

Three azulenoid sesquiterpenes (**1** – **3**) were isolated from the Antarctic gorgonian *Acanthogorgia laxa* collected by bottom trawls at −343 m. Besides linderazulene (**1**), and the known ketolactone **2**, a new brominated C_{16} linderazulene derivative (**3**) was also identified. This compound has an extra carbon atom at C(7) of the linderazulene framework. The antifouling activity of compounds **1** and **2** was assayed in the laboratory with *Artemia salina* larvae, and also in field tests, by incorporation in soluble-matrix experimental antifouling paints. The results obtained after a 45 days field trial of the paints, showed that compounds **1** and **2** displayed good antifouling potencies against a wide array of organisms. Compound **3**, a benzylic bromide, was unstable and for this reason was not submitted to bioassays. Two known cembranolides: pukalide and epoxypukalide, were also identified as minor components of the extract.

Keywords: octocorals, Acanthogorgiidae, marine natural products, azulene sesquiterpenoids, antifouling activity.

Introduction

Blue alcyonaceans contribute to the amazing color palette of the benthic invertebrate communities due to their characteristic pigments, typically derived from guaiazulene or linderazulene. These blue sesquiterpenes can be found in soft corals and gorgonians not only of tropical environments, but also in deep water species, and even in polar ecosystems.[1 – 5] Besides their contribution to the color of these organisms, several bioactivities have been reported for this class of compounds, such as cytotoxic, antimicrobial, antifungal, immunostimulatory and cell division inhibitory properties.[6] During the summer Antarctic campaigns onboard the research vessel 'Puerto Deseado', as part of an investigation on bioactive secondary metabolites from South Atlantic and Antarctic marine invertebrates, a sample of the grey-blue coral *Acanthogorgia laxa* was collected by bottom trawls at a depth of 343 m. From the extract of this organism, three sesquiterpenoids derived from guaiazulene (**1** – **3**) were isolated. In particular, compound **3** is a new brominated C_{16} derivative. It is generally believed that abundant and highly lipophilic terpenoids produced

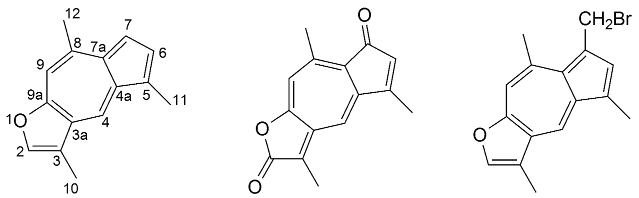
[1] Present address: Centro de Investigación de Productos Naturales y Biotecnología (CIPNABIOT), Universidad Autónoma de Chiriquí, El Cabrero, David, Chiriquí, Panamá

by benthic invertebrates may be involved in ecological interactions, such as the control of biofouling. For this reason, the toxicity of compounds **1** and **2** against *Artemia salina* larvae was tested successfully in the laboratory, and then the *in vivo* antifouling activity was assayed against a diverse array of organisms, by the preparation of experimental paints and subsequent field trials in the ocean.

Results and Discussion

Frozen tissue of *Acanthogorgia laxa* (380 g) was cut into small pieces, and exhaustively extracted with EtOH. The ethanol extract was concentrated to an aqueous suspension and then extracted with AcOEt. The organic extract was permeated through a *Sephadex LH-20* column, yielding eleven fractions. One of these fractions corresponded to an intense purple band, which yielded the main component of the extract, a purplish blue solid, which was completely characterized by HR-MS, 1D- and 2D-NMR and identified as linderazulene (**1**). Other fractions yielded the known yellow ketolactone **2**, which is derived from guaiazulene, and a previously unreported compound (**3**; *Figure 1*).

Compound **3**, a pearl-grayish amorphous solid, had a molecular formula $C_{16}H_{15}BrO$ obtained by HR-ESI-MS. An initial inspection of the ^{1}H-NMR spetrum of **3** showed great similarities with that of linderazulene **1**, especially the presence of three aromatic methyls (δ(H) 2.37 (*d*, $J = 1.3$), δ(H) 2.60 (*s*), δ(H) 3.01 (*s*)), and four characteristic signals of aromatic protons (δ(H) 6.98 (*s*), δ(H) 7.10 (*s*), δ(H) 7.37 (br. *q*), δ(H) 8.20 (*s*)). By comparison of the spectra of compounds **3** and **1**, an aromatic proton (H–C(7) in **1**) was missing in **3**, while an additional signal corresponding to a methylene group was observed (δ(H) 5.33 (br. *s*)). The downfield chemical shift of this signal suggested that it may be bound to both the bromine and the aromatic nucleus. The ^{13}C-NMR spectrum (*Table 1*) confirmed the presence of 16 carbons, and by DEPT-HSQC, these were assigned as eight quaternary aromatic carbons, four aromatic methines, three methyls and a single methylene (δ(C) 34.7). The low δ(C) of this signal together with the high δ(H) was consistent with the presence of a benzylic bromomethylene group. Since the furan and the seven membered ring of **3** were very similar to those of linderazulene, it was evident that the additional bromomethylene was located on the five membered ring, probably taking the place of the missing H–C(7). HMBC and NOESY spectra finally confirmed this tentative structure. The methylene protons had HMBCs with three aromatic carbons, and one of them was identified as C(6) (δ(C) 136.7). Additionally, NOESY correlations were observed between the methylene protons (δ(H) 5.33) with Me(12) (δ(H) 3.01) on the seven-membered ring and with H–C(6) (6.98) on the five-membered ring (*Figure 2*). In this way, the location of the bromomethylene group at C(7) was confirmed and substance **3** was identified as 7-(bromomethyl)-3,5,8-trimethylazuleno[6,5-*b*]furan, which is a new compound. An additional minor azulenoid could be identified as a C_{16} compound similar to compound **3**, probably bearing a formyl group instead of a bromomethylene group. However, due to the small amount isolated, its ^{13}C-NMR spectrum could not be fully assigned, and consequently was not claimed in the present work as a new natural product. Besides the azulenoid sesquiterpenes, a small amount (2 mg) was isolated of a mixture of the known cembranolide diterpenes pukalide and epoxypukalide, which were identified by MS and 2D-NMR directly from the mixture without further separation.[7][8]



1 **2** **3**

Figure 1. Azulenoid sesquiterpenoids from *Acanthogorgia laxa* (GPA 161).

Linderazulene (**1**), the most typical blue pigment of soft corals, was initially isolated as a product formed during the distillation over Zn of linderene, a sesquiterpenoid originally isolated from the essential

Table 1. NMR spectroscopic data of compound **3**[a]

Position	δ(H) (*J* in Hz)	δ(C)
2	7.37 (br. *q*, 1 H)	138.8
3		119.4
3a		126.1
4	8.20 (*s*, 1 H)	124.8
4a		127.5
5		137.5
6	6.98 (*s*, 1 H)	136.7
7		130.9
7a		131.9
8		140.9
9	7.10 (*s*, 1 H)	111.9
9a		158.5
10	2.37 (*d*, *J* = 1.3, 3 H)	8.0
11	2.60 (*s*, 3 H)	13.1
12	3.01 (*s*, 3 H)	27.9
13	5.33 (br. *s*, 2 H)	34.7

[a] ^{1}H: 500.13 MHz, ^{13}C: 125.13 MHz; $CDCl_3$.

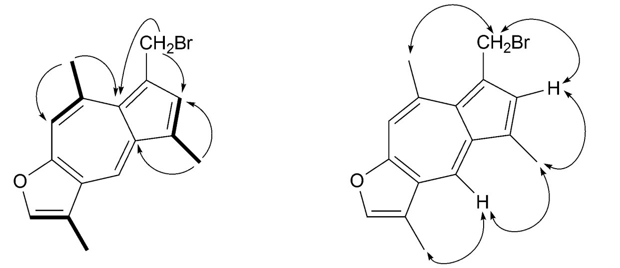


Figure 2. Key COSY (▬), HMBC (H → C), and NOESY (H ↔ H) correlations of compound **3**.

oil of the plant *Lindera strychnifolia*. This compound has been found as a natural product in the marine environment, especially in several octocoral species of the genus *Paramuricea*, while other related compounds also bearing a guaiazulene skeleton have been isolated from other genera of octocorals, such as *Anthogorgia*, *Acalycigorgia* and *Euplexaura*.[9 – 11] Linderazulene in many cases is accompanied by the yellow ketolactone **2**. Since compound **2** can be produced from **1** by oxidation, there is some controversy regarding its possible origin as an artifact.[12] There is a report that a MeOH solution of pure linderazulene, after several days on the bench, showed the presence of small amounts of compound **2**.[13] On the other hand, other authors claimed that compound **2** was detectable immediately after extraction, which pointed to a natural origin for this substance.[14] Interestingly, ketolactone **2** has also been isolated in absence of compound **1** from another gorgonian,[15] and from several terrestrial plants.[16 – 19] In the present work, compound **2** was detectable by TLC after maceration in EtOH for 1 h, as an intense yellow spot, which in the *Sephadex LH-20* column gave an intense yellow band (see photograph in *Supporting Information*). Besides that, during the subsequent work-up of the linderazulene-containing fraction, the formation of compound **2** was never detected. This points that in this case, compound **2** is not an artifact, which is not surprising since the oxidation of linderazulene can also happen in a living organism.

C_{16} Azulenoids, although quite rare, have been previously detected in gorgonians. The first example of this kind of substance was the isolation by *Scheuer* of *N*,*N*-(dimethylamino)-3-guaiazulenymethane from a deep sea gorgonian of the Paramuriceidae family. *Scheuer* also proposed a posible biosynthetic origin for this compound, as the product of a *Mannich* reaction, and subsequently synthesised the compound in that way from guaiazulene.[20] Such a derivative, in this case of linderazulene, could be a biosynthetic precursor of compound **3**, but unfortunately could not be detected in the present work. More recently, another plausible route has been proposed for the biosynthesis of C_{16} azulenoids, based in the frequent discovery of dimeric and trimeric forms of these compounds which are produced by oxidative condensation, and accompany the simple C_{16} derivatives.[21] The origin of ochracenoid A, isolated from *Anthogorgia ochracea* was proposed by an intermolecular one-carbon transfer reaction to form an additional formyl group.[22] This one-carbon transfer was originated by a complex mechanism from a dimer which was previously formed by an oxidative process. Interestingly, ochracenoid A is the dihydro analog of the previously mentioned C_{16} aldehyde derived from linderazulene, which was detected as a trace component in this work, but could not be isolated in amounts to provide a complete structural characterization. A similar pathway had been previously proposed for the biosynthesis of anthogorgienes, dimeric azulenoids from a Chinese *Anthogorgia* species.[14] This proposal is based on previous reports on the autoxidative behaviour of guaiazulene, in which similar compounds were detected.[23] However, although a similar biosynthetic pathway in this case is highly probable, it is interesting to note that in the present work no dimers of linderazulene were detected, so the biosynthetic origin of compound **3** is still unclear. Compound **3**, as other previously reported C_{16} azulenoids, was very unstable and started to decompose shortly after characterization, and for this reason was not submitted to bioassays.

Antifouling Activity

There are few previous reports on the antifouling activity of azulenoid derivatives, although it is highly probable that some of these compounds may be involved in the chemical defense of gorgonians. There are no reports of positive antifouling activity for linderazulene, while compound **2** was reported to inhibit the larval settlement of the barnacle *Balanus amphitrite* with an EC_{50} of 6.69 µg/mL.[14] A compound closely related to **1**, 8,9-dihydrolinderazulene, displayed cytotoxicity against several tumor cell lines: PC-3, HCT-116 and MCF-7/ADR with IC_{50} values of 9.46, 14.49 and 16.06 mg/mL. However, when this compound was tested for larval toxicity against *Artemia salina*, or for antifouling activity against *B. amphitrite*, no activity was observed.[6] The development of biofouling is a complex process, which comprises several stages, in which compounds with different bioactivity profiles may be useful for its control. For example, compounds with antibiotic activity can inhibit the formation of the primary bacterial film, one of the first stages in the process of biofouling. On the other hand, compounds that may be toxic to larvae can inhibit the process at a later stage. The laboratory assays most commonly used for antifouling activity, as the larval settlement of *B. amphitrite*, generally study the action of the tested compounds on one species. However, the fouling community is much more varied, and includes species from different *phyla*, so these results should be interpreted with care, since they may be not very realistic. In the present work, the antifouling activities of compounds **1** and **2** were studied using a multi-target approach: first in the laboratory using a larval toxicity assay against *A. salina* as a preliminary test, and then in realistic field trials by incorporation of the compounds in the formulations of soluble-matrix antifouling paints. In this way, the effects of the compounds on the complete fouling community were evaluated after field trials at Mar del Plata harbor.

The brine shrimp assay (*A. salina*) was used for many years as a preliminary test for cytotoxicity, although in recent years, with the development of cell-line tests, its use has declined. However, this test is still a valuable tool for the study of larval toxicity and, consequently, for antifouling activity.[24] Since compounds **1** and **2** displayed activity against *Artemia* nauplii, they were considered good candidates for field trials with soluble matrix paint.

Soluble matrix paints are one of the main technologies for antifouling coatings. In these paints, the matrix is based on colophony resin, and the slightly basic pH of seawater produces a controlled dissolution of the resinic acids, with a slow and continuous release of the biofoulants as well.[25] Antifouling paints were prepared with the addition of one of the tested compounds; acrylic tiles were then painted and tested in the sea at Mar del Plata harbor. Although this assay was originally designed to test the efficacy of antifouling coatings, it is one of the most realistic tests for antifouling activity, since the abiotic and biotic factors are not regulated, and the test surfaces are offered to a diverse fouling community, which has various settlement trends.[26][27] Settlement of foulers on the experimental paints occurs under natural conditions of flow and diffusion while being exposed to a natural supply of larvae and algal spores.

The results of the *A. salina* toxicity assays are shown in *Figure 3*. After 24 h, increasing concentrations of linderazulene **1** and ketolactone **2** affected *Artemia* nauplii survival. ANOVA analysis showed significant differences among treatments and controls ($P < 0.05$), and this response was dose-dependant. Additionally, no significant differences between controls were observed. LC_{50} obtained by *Probit* analysis was 2.681 μg/mL (12.8 μM) for linderazulene **1** and 5.253 μg/mL for ketolactone **2**.

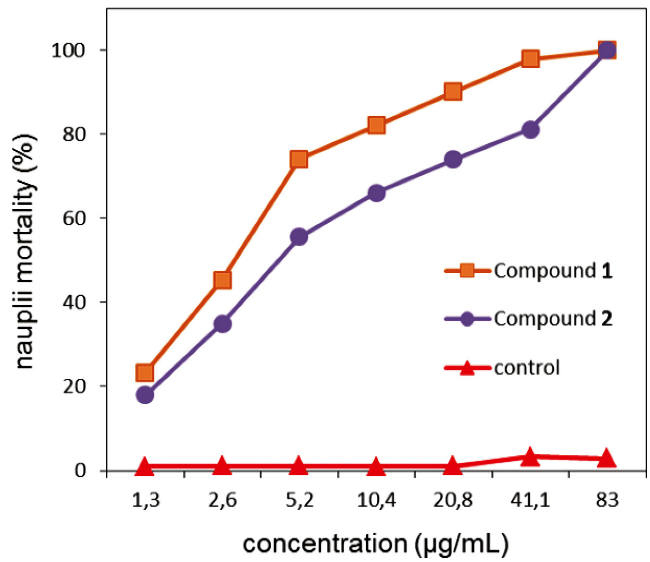


Figure 3. Larval toxicity of compounds **1** and **2** against *A. salina* nauplii.

The results of the field trials with the experimental paints are shown in *Figure 4*, expressed in coverage percentage for each fouling species. The main macrofouling species of Mar del Plata harbor were strongly affected by the coatings containing compounds **1** or **2**. After 45 days of exposure, both paints completely inhibited the settlement of the red alga *Griffithsia* sp., the calcareous tube-worm *Hydroides* sp., the sandtube builder *Polydora* sp. and the colonial ascidian *Botryllus* sp. The settlement of bryozoan colonies of *Bugula* sp.

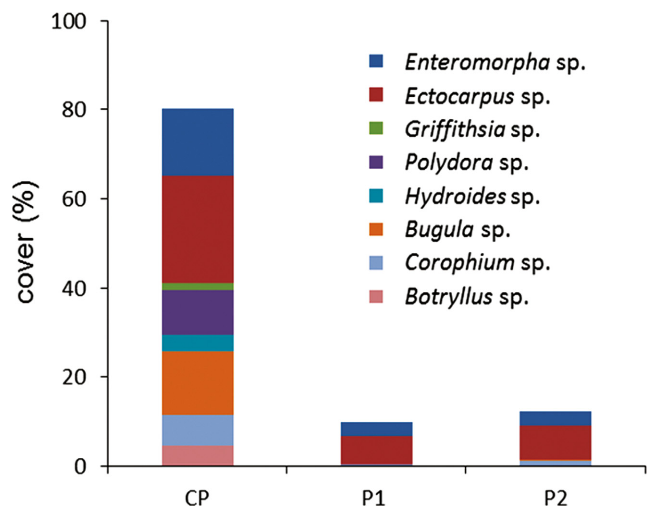


Figure 4. Macrofouling percentage cover after 45 days exposure. P1: Antifouling paint containing linderazulene; P2: antifouling paint containing ketolactone.

was also completely inhibited by the paint with linderazulene, but only reduced by the paint containing ketolactone **2** ($P < 0.05$). The cover percentages of green and brown algae (*Enteromorpha* sp. and *Ectocarpus* sp., respectively) and the settlement of *Corophium* sp. tubes, were also strongly reduced by both coatings.

Conclusions

This is the first study of the chemical constituents of *A. laxa*. Previous investigations on other species of this genus from other regions reported the presence of xenicane diterpenes and polihydroxy sterols, but not of sesquiterpenes.[28][29] Compounds **1** – **3** are the first reported azulenoids for the genus *Acanthogorgia*, and compound **3** is the first brominated C_{16} azulenoid. The results obtained from the antifouling activity assays clearly show that azulenoid sesquiterpenoids are involved in the control of biofouling by marine invertebrates. As always, the marine environment provides knowledge and inspiration for the development of additives for the control of biofouling. In the present work, it was also demonstrated that azulenoids can be environmentally friendly natural additives for marine paints. Although the industrial use of marine natural products obtained from fragile and slow-growing marine invertebrates is out of the question due to ecological reasons, there are other options, which are ecologically sustainable. Historically, several kinds of azulenoid pigments have been obtained by Zn distillation of abundant plant natural products and essential oils, a process that may be amenable for scaling-up.[30 – 32] On the other hand, ketolactone **2** has been also isolated from abundant terrestrial plants, among them, several species of *Curcuma*.[18][19] In these plants, compound **2** is probably originated in the oxidation of more abundant sesquiterpene lactones, a process that can be reproduced in the laboratory and possibly scaled-up. These methodologies offer ecologically sustainable alternatives for the large-scale production of these potentially useful antifouling compounds.

Experimental Section

General

Gel permeation chromatography was performed in MeOH, using *Sephadex LH20* (*GE Healthcare*). HPLC separations were performed using a *Thermo Separations SpectraSeries P100* pump, a *Thermo Separations Refractomonitor IV RI* detector connected to a *Thermo Separations SpectraSeries UV 100* detector, with simultaneous UV (220 nm) and RI detection. An *YMC RP-18* (5 μm, 20 mm × 250 mm) column working at a flow rate of 5 mL/min was used for HPLC separations. All solvents were HPLC grade. UV Spectra were recorded in MeOH on a *Hewlett-Packard 8452* spectrometer; λ_{max} (ε) in nm. Infrared spectra were obtained (film on KBr) on a *Nicolet-Magna 550* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. All NMR spectra were recorded in $CDCl_3$ using the signals of residual non-deuterated solvent as internal reference on a *Bruker Avance II 500* MHz spectrometer operating at 500.13 MHz for ^1H and 125.13 MHz for ^{13}C; δ in ppm, J in Hz. All 2D-NMR experiments (COSY, DEPT-HSQC, HMBC, NOESY) were performed using standard pulse sequences. High resolution mass spectra were recorded on a *Bruker micro-TOf-Q* instrument, using ESI or APCI ionization; in *m/z*.

Animal Material

Samples of the grey-blue gorgonian *Acanthogorgia laxa* were collected by bottom otter trawls (−343 m) onboard the research vessel 'Puerto Deseado' (CONICET) in Antarctic waters. The sampling station was located at 64°41.5′ S, 63°1.6′ W, South Shetland islands. The biological material was frozen onboard (−20 °C), transported to the laboratory, and kept frozen until processed. A voucher specimen was identified by us (*C. D. P.*, *R. C.*) and is kept at the cnidarian collection of the Anthozoan Research Group (GPA) at the Academic Center of Vitoria, University of Pernambuco (Brazil) with the number GPA 161. An additional voucher specimen was deposited with the 'Museo Argentino de Ciencias Naturales Bernardino Rivadavia' (Buenos Aires, Argentina) with the number MACN-In 41255.

Extraction and Isolation

Frozen samples of *A. laxa* (380 g) were triturated with EtOH (1 L) in a blender, and the residue was extracted twice more with EtOH (500 mL each). The combined extracts were concentrated under reduced pressure to obtain an aqueous suspension, which was re-extracted with AcOEt. Evaporation of the organic phase gave 2.80 g of a purple-brown syrup. The extract was permeated on *Sephadex LH20* (4 × 120 cm, MeOH), to afford eleven fractions. *Fraction 7* was an intense purple band (see photograph in *Supporting Information*) that yielded 123.7 mg of pure linderazulene **1**. *Fractions 9* – *10* were pooled and, by evaporation yielded 4 mg of a grayish amorphous solid which was identified as compound **3**. *Fraction 5* (118 mg) was re-permeated on *Sephadex LH-20* to obtain 54 mg of a terpenoid-rich fraction which was subjected to HPLC on reversed phase (MeOH as eluant), to obtain

22.3 mg of ketolactone **2** and a fraction (2 mg) which by HR-ESI-MS, 1D- and 2D-NMR spectra, and comparison with literature data, was identified as a 2:1 mixture of pukalide and epoxypukalide. Finally, *Fraction 6* (28 mg) was purified by HPLC (MeCN/H_2O 85:15) to yield an additional 8 mg of ketolactone **2** and trace amounts of a C_{16} aldehyde which could not be fully characterized by ^{13}C-NMR due to lack of sample.

7-(Bromomethyl)-3,5,8-trimethylazuleno[6,5-*b*]-furan (**3**). Pearl-gray amorphous solid. UV (MeOH): 250 (1267), 285 (1100), 574 (83), 581 (102). IR (film on KBr): 1468, 1390, 810. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz): see *Table 1*. HR-ESI-MS: 325.0206 ($[M + Na]^+$, $C_{16}H_{15}BrNaO^+$; calc. 325.0203).

Antifouling Activity Assays

Larval Toxicity Assay. Compounds **1** and **2** were evaluated for toxic effect against *Artemia salina* nauplii.[33] Stock solutions were prepared by dissolving 1 mg of each compound in DMSO. From these stock solutions, a series of dilutions in artificial seawater were obtained with the following concentrations: 1.3, 2.6, 5.2, 10.4, 20.8, 41.6, and 83.2 μg/mL. Controls were prepared with artificial seawater + DMSO. Brine shrimp eggs were hatched in artificial seawater and after 24 h the phototrophic nauplii were collected by pipette from the light side. Toxicity tests were performed by adding thirty *Artemia salina* nauplii to each well of 24-Multiwell plates containing 2 mL of the different test solutions. Each treatment was replicated three times. The plates were incubated for 24 h at room temperature and then, dead nauplii were counted. Finally, fifty lethal concentration (LC_{50}) was obtained by *Probit* analysis.

Soluble Matrix Paint Preparation

Soluble matrix antifouling paint was prepared by dissolving colophony (WW rosin) and oleic acid in a xylene/methyl isobutyl ketone mixture (1:1) using a high-speed disperser. Colophony was used as binder and oleic acid as plasticizer. The ball mill (1.0 L jars) was loaded with this mixture ('vehicle') and pigments (zinc oxide and calcium carbonate), and dispersed for 24 h. Then, the paint was filtered and fractionated in three parts, one for linderazulene (**1**), another for the ketolactone **2**, and the remaining one for control. For treatments, pure compounds (linderzulene or ketolactone) were dissolved in DMSO and incorporated to the paint matrix at a final concentration of 125 mg/L of paint. Finally, the same amount of DMSO that was used for the treatments was added to the control paint. Quantitative paint formulation was previously described.[27]

Field Trials

Sandblasted acrylic panels (4 × 12 cm), which had been previously degreased with toluene, were painted for field trials. Three replicates were employed for each treatment and controls (base paint, *i.e.*, paint without compounds). The painted panels were hung from frames, and submersed at 50 cm below water line for 45 days during summer months at the marina of 'Club de Motonáutica' (Mar del Plata harbor, Argentina). The settlement of fouling organisms was measured as percentage cover on each panel using a dot-grid estimated method.[34]

Statistical Analyses

All statistical analyses were performed with SPSS Statistics software. The differences between treatment and control were determined by one-way analysis of variance (ANOVA) followed by *Tukey* post hoc test. Differences were considered significant at $P < 0.05$. Estimations of LC_{50} were calculated through *Probit* analysis.

Supplementary Material

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.201700425.

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 2010 No. 1808, PICT 2014-2063). We thank Dr. *Gabriela Cabrera* (*UMYMFOR–CONICET*) for recording the mass spectra and *Eng. José Gallardo* and *Lic. Gernot Eskuche* (*UMYMFOR–CONICET*) for the NMR spectra. *L. P. P. C.* thanks *SENACYT-IFARHU* (Panamá) for a doctoral fellowship. *L. S.* thanks the crew of RV 'Puerto Deseado'; participation in the research cruise was partially supported by *IAA* (Instituto Antártico Argentino), *INIDEP* and *PICT* 2013-0629. Research at the Federal University of Pernambuco was supported by a grant from *CNPq-Brazil* (408934/2013-1).

Author Contribution Statement

Laura P. Patiño Cano and *Rodrigo Quintana Manfredi* isolated and identified the compounds from *A. laxa*.

Jorge A. Palermo was the director of the project and wrote the manuscript. *Laura Schejter* organized the onboard activities, collected the marine organisms and performed initial identifiction of invertebrates. *Miriam* Pérez, *Mónica García* and *Guillermo Blustein* performed the bioassays for antifouling activity. *Carlos D. Pérez* and *Ralf Cordeiro* were in charge of the taxonomic identification of the cnidarian samples.

References

[1] J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. G. Munro, M. R. Prinsep, 'Marine Natural Products', *Nat. Prod. Rep.* **2016**, *33*, 382 – 431, and previous reviews.

[2] S. Sakemi, T. Higa, '2,3-Dihydrolinderazulene, a New Bioactive Azulene Pigment from the Gorgonian *Acalycigorgia* sp.', *Experientia* **1987**, *43*, 624 – 625.

[3] M. OChi, K. Kataoka, A. Tatsukawa, H. Kotsuki, K. Shibata, 'Gorgiabisazulene and Gorgiagallylazulene, Two New Guaiazulenoid Pigments from a Gorgonian *Acalycigorgia* sp.', *Chem. Lett.* **1993**, 2003 – 2006.

[4] N. Srinivasa Reddy, J. K. Reed, R. E. Longley, A. E. Wright, 'Two New Cytotoxic Linderazulenes from a Deep-Sea Gorgonian of the Genus *Paramuricea*', *J. Nat. Prod.* **2005**, *68*, 248 – 250.

[5] I. I. Kapustina, A. I. Kalinovskii, B. B. Grebnev, A. S. Savina, V. A. Stonik, 'Sesquiterpenoids from the Far-Eastern Gorgonaria *Stenogorgia* sp.', *Chem. Nat. Compd.* **2009**, *45*, 916 – 917.

[6] M. Chen, L. Han, Y. Wang, X. L. Zhang, C. Y. Wang, 'A New Sesquiterpene from the Hainan Gorgonian *Menella kanisa* Grassoff', *Nat. Prod. Res.* **2014**, *28*, 1147 – 1151.

[7] M. G. Missakian, B. J. Burreson, P. J. Scheuer, 'Pukalide, a Furanocembranolide from the Soft Coral *Sinularia abrupta*', *Tetrahedron* **1975**, *31*, 2513 – 2515.

[8] M. B. Ksebati, L. S. Ciereszko, F. J. Schmitz, '11β,12β-Epoxypukalide, a Furanocembranolide from the Gorgonian *Leptogorgia setacea*', *J. Nat. Prod.* **1984**, *47*, 1009 – 1012.

[9] S. Imre, R. H. Thomson, B. Yalhi, 'Linderazulene, a Naturally Occurring Pigment from the Gorgonian *Paramuricea chamaeleon*', *Experientia* **1981**, *37*, 442 – 443.

[10] E. Manzo, M. L. Ciavatta, M. P. Lopez Gresa, M. Gavagnin, G. Villani, C. G. Naik, G. Cimino, 'New Bioactive Hydrogenated Linderazulene-Derivatives from the Gorgonian *Echinogorgia complexa*', *Tetrahedron Lett.* **2007**, *48*, 2569 – 2571.

[11] N. Penez, G. Culioli, T. Pérez, J. F. Briand, O. P. Thomas, Y. Blache, 'Antifouling Properties of Simple Indole and Purine Alkaloids from the Mediterranean Gorgonian *Paramuricea clavata*', *J. Nat. Prod.* **2011**, *74*, 2304 – 2308.

[12] M. K. W. Li, P. J. Scheuer, 'A Guaianolide Pigment from a Deep-Sea Gorgonian', *Tetrahedron Lett.* **1984**, *25*, 2109 – 2110.

[13] B. Alpertunga, S. Imre, H. J. Cowe, P. J. Cox, R. H. Thomson, 'A Photo Artefact from Linderazulene', *Tetrahedron Lett.* **1983**, *24*, 4461 – 4462.

[14] D. Chen, S. Yu, L. van Ofwegen, P. Proksch, W. Lin, 'Anthogorgienes A – O, New Guaiazulene-Derived Terpenoids from a Chinese Gorgonian *Anthogorgia* Species, and Their Antifouling and Antibiotic Activities', *J. Agric. Food Chem.* **2012**, *60*, 112 – 123.

[15] L. Xue, P. L. Li, Z. Liang, X. L. Tang, G. Q. Li, 'Sesquiterpenoids and Steroids from Gorgonian *Echinogorgia sassapo reticulate*', *Biochem. Syst. Ecol.* **2014**, *57*, 48 – 51.

[16] V. U. Ahmad, S. Yasmeen, Z. Ali, M. Ali Khan, M. I. Choudhary, F. Akhtar, G. Abbas Miana, M. Zahid, 'Taraxacin, a New Guaianolide from *Taraxacum wallichii*', *J. Nat. Prod.* **2000**, *63*, 1010 – 1011.

[17] K. Kawazoe, Y. Tsubouchi, N. Abdullah, Y. Takaishi, H. Shibata, T. Higuti, H. Hori, M. Ogawa, 'Sesquiterpenoids from *Artemisia gilvescens* and an Anti-MRSA Compound', *J. Nat. Prod.* **2003**, *66*, 538 – 539.

[18] C.-M. Zhang, P.-H. Fan, M. Li, H.-X. Lou, 'Two New Sesquiterpenoids from the Rhizomes of *Curcuma xanthorrhiza*', *Helv. Chim. Acta* **2014**, *97*, 1295 – 1300.

[19] M. G. Phan, T. T. Nhan Tran, T. S. Phan, K. Matsunami, H. Otsuka, 'Guaianolides from *Curcuma kwangsiensis*', *Phytochem. Lett.* **2014**, *9*, 137 – 140.

[20] M. K. W. Li, P. J. Scheuer, '*N, N*-Dimethylamino-3-Guaiazulenylmethane from a Deep-Sea Gorgonian', *Tetrahedron Lett.* **1984**, *25*, 4707 – 4708.

[21] Y. Seo, J. R. Rho, N. Geum, J. B. Yoon, J. Shin, 'Isolation of Guaianoid Pigments from the Gorgonian *Calicogorgia granulosa*', *J. Nat. Prod.* **1996**, *59*, 985 – 986.

[22] J. J. Zheng, C. L. Shao, M. Chen, L. S. Gan, Y. C. Fang, X. H. Wang, C. Y. Wang, 'Ochracenoids A and B, Guaiazulene-Based Analogues from Gorgonian *Anthogorgia ochracea* Collected from the South China Sea', *Mar. Drugs* **2014**, *12*, 1569 – 1579.

[23] S. Takekuma, Y. Matsubara, H. Yamamoto, T. Nozoe, 'Autoxidation of Solid Guaiazulene and of the Solution in DMF in the Presence of Base or Acid: A Comparative Study of the Product Distribution', *Bull. Chem. Soc. Jpn.* **1988**, *61*, 475 – 481.

[24] J. Castritsi-Catharios, N. Bourdaniotis, G. Persoone, 'A New Simple Method with High Precision for Determining the Toxicity of Antifouling Paints on Brine Shrimp Larvae (*Artemia*): First Results', *Chemosphere* **2007**, *67*, 1127 – 1132.

[25] M. Lejars, A. Margaillan, C. Bressy, 'Fouling Release Coatings: A Non-Toxic Alternative to Biocidal Antifouling Coatings', *Chem. Rev.* **2012**, *112*, 4347 – 4390.

[26] M. Pérez, M. García, M. Sánchez, M. Stupak, M. Mazzuca, J. A. Palermo, G. Blustein, 'Effect of Secochiliolide Acid Isolated from the Patagonian Shrub *Nardophyllum bryoides* as Active Component in Antifouling Paints', *Int. Biodeterior. Biodegr.* **2014**, *89*, 37 – 44.

[27] M. Pérez, M. Sánchez, M. Stupak, M. García, M. T. Rojo de Almeida, J. C. Oberti, J. A. Palermo, G. Blustein, 'Antifouling Activity of Celastroids Isolated from *Maytenus* Species, Natural and Sustainable Alternatives for Marine Coatings', *Ind. Eng. Chem. Res.* **2014**, *53*, 7655 – 7659.

[28] E. Manzo, M. L. Ciavatta, M. Gavagnin, G. Villani, C. Quaranta, L. D'Souza, G. Cimino, 'A New Xenicane Norditerpene from the Indian Marine Gorgonian *Acanthogorgia turgida*', *Nat. Prod. Res.* **2009**, *23*, 1664 – 1670.

[29] W. Zhang, Y. W. Guo, E. Mollo, A. Fontana, G. Cimino, 'Acanthovagasteroids A – D, Four New 19-Hydroxylated Steroids from the South China Sea Gorgonian *Acanthogorgia vagae Aurivillius*', *J. Nat. Prod.* **2004**, *67*, 2083 – 2085.

[30] K. Takeda, W. Nagata, 'Components of the Root of *Lindera strychnifolia* Vill. V. Azulenes Isolated from Linderene by Zn Dust Distillation', *Pharm. Bull.* **1953**, *1*, 164 – 169.

[31] J. Romo, A. Romo de Vivar, W. Herz, 'Constituents of *Helenium* Species XIV. The Structure of Mexicanin E', *Tetrahedron* **1963**, *19*, 2317 – 2322.
[32] U. Mölleken, V. Sinwell, K. H. Kubeczka, 'The Essential Oil Composition of Fruits From *Smyrnium perfoliatum*', *Phytochemistry* **1998**, *47*, 1079 – 1083.
[33] B. N. Meyer, N. R. Ferrighi, J. Putnam, L. B. Jacobsen, D. E. Nichols, J. L. McLaughlin, 'Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents', *Planta Med.* **1982**, *45*, 31 – 34.
[34] M. S. Foster, C. Harrold, D. D. Hardin, 'Point Versus Photo Quadrat Estimates of the Cover Sessile Marine Organisms', *J. Exp. Mar. Biol. Ecol.* **1991**, *146*, 193 – 203.

Received September 15, 2017
Accepted October 26, 2017

