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Effect of secochiliolide acid isolated from the Patagonian shrub *Nardophyllum bryoides* as active component in antifouling paints

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ABSTRACT

Environmental concerns about the use of toxic antifoulants have led to an increased interest in the development of new alternatives. So far, most of the antifouling natural products have been obtained from marine organisms. However, some secondary metabolites from terrestrial plants could be promising antifoulant candidates. The antifouling performance of secochiliolide acid, the main component isolated from *Nardophyllum bryoides* ethanolic extract, was evaluated for inclusion in rosin-based coatings.

Field testing was conducted during the summer months at Mar del Plata harbor, Argentina. The results indicated that secochiliolide acid-based paints completely inhibited the settlement of *Bugula neritina* colonies, *Polydora* sp., *Hydroides elegans*, *Corophium* sp. and solitary ascidians, and also reduced the attachment of some algae as *Enteromorpha intestinalis* and *Ectocarpus* sp. In addition, a lower density and diversity of microfouling species was registered.

These results highlighted the importance of terrestrial plants as a sustainable source of potential environmentally friendly antifoulants.

1. Introduction

Marine biofouling of ship hulls is an age-old problem, since the surface condition of the hull is of primary importance in the performance of marine vessels. It is well established that biofouling on ships increases the surface roughness of the hull which in turn causes additional frictional resistance, reduces maneuverability and efficiency, increases fuel consumption and decreases top speed (Lewthwaite et al., 1985; Leer-Andersen and Larsson, 2003; Schultz, 2007). These combined factors result in increased fuel costs and a higher frequency of dry-docking with associated economic losses (Schultz et al., 2011). There are also possible ecological consequences of biofouling due to the inadvertent introduction of invasive foreign species (Minchin and Gollasch, 2003; Floerl et al., 2004, 2005; Lejars et al., 2012). Many submerged structures are consequently protected by biocidal, antifouling coatings in order to minimize the effects due to the colonization of micro and macroorganisms (Evans, 1999).

Since the use of biocides in antifouling paints (in particular organotins) is becoming increasingly restricted, a significant research effort has been focused on the development of environmentally benign technologies to control fouling, of which one of the most promising is the use of non-toxic and potentially biodegradable natural products (Maréchal and Hellio, 2009; Thomas and Brooks, 2010; Blihoghe et al., 2011).

Marine benthic organisms are constantly exposed to colonization by bacterial communities and larvae of fouling organisms. Some of these organisms have developed various strategies to counteract the settlement of fouling organisms, such as the production of antifouling chemicals and/or physical defenses (Tan et al., 2010). To date, several examples of natural products with

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antifouling activity have been isolated from a variety of marine organisms, including marine bacteria, algae, seagrasses, sponges, corals, bryozoans, ascidians, etc. (Clare, 1996; Hellio et al., 2000; Rittschof, 2001; Da Gama et al., 2002; Steinberg and de Nys, 2002; Faimali et al., 2003; Angarano et al., 2007; Tsoukatou et al., 2007; Sjögren et al., 2008; Feng et al., 2009; Raveendran and Limna Mol, 2009; Villa et al., 2010; Qian and Xu, 2012; Nguyen et al., 2013). In fact, many of the natural products that have been discovered from marine organisms on the basis of their pharmacological activity may play an ecological role for their source species in the marine environment, in many cases acting as natural antifoulants. However, the production of these bioactive substances from marine sources on a large scale is a big challenge for the antifouling technology, because, to date, most of these metabolites have been isolated in low yields from delicate and slow-growing marine organisms such as corals, sponges and other invertebrates which cannot be harvested on a commercial scale without environmental harm (Rittschof, 2001). Mariculture of these marine invertebrates is not an easy task, and under different environmental conditions the production of bioactive metabolites may vary substantially. All these issues pose an almost unsolvable sustainability problem for the large-scale production of natural antifoulants of marine origin.

For this reason, an additional effort has to be made in the search of natural antifoulants from more sustainable resources such as abundant and easy collectable terrestrial plants. Comparatively little attention has been given to terrestrial plants in the search for natural products which may act as antifoulants, and only a few antifouling compounds have been reported from these sources (Yamashita et al., 1989; Hyodo et al., 1992; Sawant and Wagh, 1994; Sawant et al., 1995; Göransson et al., 2004; Angarano et al., 2007; Pérez et al., 2007; Chen et al., 2008; Zhou et al., 2009; Ovesen et al., 2011). Plant natural products from abundant and widely distributed species represent an attractive and sustainable source of new bioactive compounds. Terrestrial plants produce secondary metabolites that exhibit a variety of biological activities, many of which are of considerable significance to humans (Sánchez et al., 2010). It is worth noting that some plant extracts have shown antifouling activity, e.g. *Quercus dentate* (Yamashita et al., 1989), *Xanthium strumarium* (Harada et al., 1985), *Eucalyptus resinifera* (Hyodo et al., 1992), *Eucalyptus grandis* (Singh et al., 1996), *Eucalyptus rubida* (Yamashita et al., 1986) and *Zingiber officinale* (Etoh et al., 2002) on mussel byssal thread formation, *Acacia pennata* and *Barringtonia acutangula* on some diatoms and invertebrates (Sawant and Wagh, 1997), *Schinopsis* sp. tannin and tannate (Stupak et al., 2003; Pérez et al., 2007; Blustein et al., 2009) and some Chinese herbs on barnacle settlement (Feng et al., 2009). Additionally, some common plants as *Capsicum* sp. (pepper), *Allium* sp. (onion) and *Derris scandens* (hog creeper) restrain the attachment of cirripede larvae and bacteria or inhibit their growth (Sawant et al., 1995; Xu et al., 2005a,b; Lin et al., 2009). This is no surprise, considering the abundance of antibiotic or cytotoxic secondary metabolites in plant extracts that could affect either biofilm formation as the settlement of larvae.

In this context, *Nardophyllum bryoides* (*Chiliotricum* group, Asteraceae), a widely distributed shrub in Argentinean and Chilean Patagonia and the Andes (Jakupovic et al., 1986; Bonifacino, 2005) was selected to evaluate its possible antifouling activity. In previous studies, some metabolites isolated from this species have shown moderate cytotoxic activity against human pancreatic adenocarcinoma cell lines (Sánchez et al., 2010) and strong trypanocidal effect (Siless et al., 2013). Also, the extracts from another species of this genus, *Nardophyllum armatum*, showed antioxidant, antibacterial, antirheumatic and antifungal activity. In addition, digestive, antitussive and febrifuge properties have been reported for this species (D'Almeida et al., 2007; Barboza et al., 2009; D'Almeida et al., 2011).

The crude ethanolic extract of *N. bryoides* was included in the formulation of a soluble-matrix antifouling paint, which was tested in field trials in Mar del Plata harbor. In this type of paints, water diffusion within the matrix could dissolve any water-soluble components and lead to (i) the diffusion of active species out of the coating (release) and/or (ii) the dissolution of the soluble-matrix paint by the slightly alkaline pH of seawater (erosion). These two mechanisms lead to the release of bioactive species and the renewal of the surface, respectively (Lejars et al., 2012). The release rate of insoluble active molecules from soluble-matrix paints is often controlled by the erosion rate of the immersed coating. The promising results obtained with the paint containing the crude extract of *N. bryoides* led us to also test an enriched fraction of this extract, and finally to the identification of secochiliolide acid (**1**), the main component of the extract, as a promising antifouling substance.

2. Material and methods

2.1. General experimental procedures for extraction and isolation

Solvents were distilled for chromatography. NMR spectra were recorded on Bruker AC-200 (200.13 MHz) and Bruker Avance II (500.13 MHz) spectrometers, using the signals of residual non-deuterated solvents as an internal reference. All 2D NMR experiments (COSY, DEPT-HSQC, HMBC, and NOESY) were performed using standard pulse sequences. HRMS were acquired on a Bruker micrOTOF-Q II spectrometer. TLC was carried out on Merck Sílicagel 60 F254 plates. TLC plates were sprayed with 2% vanillin in concentrated H_2SO_4. Merck Silicagel (230–400 mesh) was used for column chromatography. Sephadex LH-20 was obtained from Pharmacia Inc.

2.2. Plant material

Specimens of *N. bryoides* were collected at Departamento de Escalante, Province of Chubut (Argentina), in February 2008 (summer). A voucher specimen (HRP6865) was identified by María Elena Arce (Universidad Nacional de la Patagonia San Juan Bosco, Argentina) and was stored at the Herbario Regional Patagónico, Universidad Nacional de la Patagonia San Juan Bosco.

2.3. Extraction, fractionation and compound identification

Ground aerial parts of fresh plant material (1000 g) were extracted exhaustively with ethanol (3 times, 24 h each) at room temperature (20 ± 1 °C), and the extract was evaporated at reduced pressure to yield a syrupy residue (90 g). This residue was partitioned between MeOH: H_2O (9:1) and cyclohexane. The yield of polar subextract was 5.8 g per 100 g of fresh plant material. The polar subextract (NOP) was chosen for the experiments because, by preliminary chromatographic analysis, it had a higher content of secondary metabolites with the presence of some major components. In contrast, the lipophilic subextract (NOL) showed no major components and, by chromatographic and spectroscopic inspection (NMR), was not interesting from a chemical point of view. The polar subextract (NOP) was concentrated to an aqueous suspension and then partitioned between EtOAc and 10% aqueous NaOH. The basic aqueous phase was then acidified (pH = 3) by addition of 2 M HCl, and extracted three times with EtOAc. This last organic phase was evaporated up to dryness, giving an acidic component-enriched fraction (NOPab). Chromatographic and spectroscopic inspection