Biologically active substances from water invertebrates: a review

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ABSTRACT: Some species of invertebrates especially bryozoans (Bryozoa syn. Ectoprocta) and marine sponges (Porifera) are very important sources of pharmacologically exploitable compounds. These substances are probably produced to protect themselves from fish predators and may be an advantage in competition. The real sources of compounds with these antipredatory effects are probably not marine invertebrates themselves, but microscopic symbionts or food which they feed on. Bryostatins from bryozoan species *Bugula neritina* are produced by a bacterial symbiont called *Candidatus Endobugula sertula*. They have significant anti-cancer effects, but also other therapeutic benefits. Compounds with the structure of bryostatins were also discovered in some other invertebrates. Sponges are a source of many compounds, e.g., ara-A (vidarabine), manzamine, lasonolides, spongistatins, peloruside and others with antimicrobial, anti-cancer, immunosuppressive and similar activities. Other important sources of compounds with medical effects are tunicates (Tunicata syn. Urochordata) and some snails (Mollusca). One drug was developed from tunicates – Yondelis against refractory soft-tissue sarcomas. Certain other drugs originate from snails: e.g., prialt, which acts against chronic pain in spinal cord injury.

Keywords: bioactive substances; antipredatory effect; competition; bryozoan; bryostatin; *Bugula neritina*; symbiont; marine sponges; isocyano terpenes; tunicates; snails

Contents

1. Introduction

Many groups of invertebrates produce specific substances that serve them as (1) defensive agents against predators, parasites and infections or (2) chemicals for intraspecific and interspecific communication. Some of these compounds have interesting properties which could potentially be exploited in pharmacology. Bryostatins are among the most important compounds and are produced by marine organisms from the phyllum Bryozoa (Ectoprocta). Bryostatins are perhaps the best example of bioactive compounds from bryozoans (Prinsep et al. 2004). They primarily have antican-

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cer effects (Dunlap et al. 2007) and constitute one family of protein kinase C modulators (Trindade-Silva et al. 2010).

The research on bryostatins is very extensive and complex. It can be expected that these substances will be tested on various animal species or even used in veterinary medicine. This review aims to highlight the basic directions of research on these substances.

Due to the increasing interest in these substances it is likely that the veterinary sector will come into contact with them more often, whether with regard to testing on various animal species or their possible use.

Dunlap et al. (2007) reported that 22 marine natural products or chemical analogues thereof, are now in clinical trials. The majority of these have potential anticancer effects; others have potential benefits in therapeutic areas of inflammation, pain, asthma and Alzheimer's disease. Sponges and bryozoans stand out from the other phyla by having a very high occurrence of bioactive compounds (Prinsep et al. 2004).

New drug development from marine natural products is currently more important than ever before. New technologies in analytical spectroscopy enable the discovery of new molecules in a few micrograms of an organism, which is a fraction of the material required 10 years ago. It is possible to manipulate biosynthetic pathways even in microbiotic symbionts, which are often the true source of potential drugs. Most genes responsible for the biosynthesis of drugs have been indentified and sequenced (Molinski et al. 2009). At present, scientists are working on the development of permanent cell lines of marine invertebrates (Odintsova et al. 2011).

2. Biological effects of specific substances

Although sessile marine invertebrates (sponges, bryozoans, ascidians and others) lack significant physical defences (Schmidt 2005; Ebada et al. 2010), they are able to thrive in highly competitive environments (Schmidt 2005). They are probably protected by chemical defences and some of their compounds can be harvested for therapeutic use (Schmidt 2005; Ebada et al. 2010). Larval chemical defence of benthic marine invertebrates plays an importance role. These larvae can be big, colourful and active in benthic habitats teeming with planktivorous fishes (Lopanik et al. 2004a). Many marine natural products play the key role in the chemical defence of many marine animals and in some cases they can influence the composition of the entire ecosystem (Paul et al. 2007).

Most bryozoans are vulnerable to predation or infection (Prinsep et al. 2004). The highly vulnerable larval stage of *Bugula neritina* is chemically defended from fish predation by bryostatins (Lopanik et al. 2004a). Lopanik et al. (2004b) isolated three bryostatins from the larvae of *B. neritina*, bryostatin 10, bryostatin 20, and a yet uncharacterised bryostatin, that was unpalatable to fish. These deterrent bryostatins represent an important example from the marine environment of a microbial symbiont producing an antipredatory defence for its host (Lopanik et al. 2004a). Bryostatin 10 is most abundant in larvae, probably for its antipredatory effects, but it will be difficult to determine exactly the biochemical mechanisms by which bryostatins deter predators.

The results of the study of Lopanik et al. (2006) suggest that the different life stages of *B. neritina* employ alternate defensive strategies. Mobile, short-lived larvae and early-stage juveniles are defended from predators by means of bryostatins; in contrast, older juveniles and adults, which are clonal and relatively long-lived, may be defended by high levels of structural material (i.e., chitin and carbonate). It is possible that high concentrations of bryostatins in some zooid colonies can reduce predation too (Winston and Woollacott 2008). Predation pressure on the vulnerable larval stage of *B. neritina* appears in part to have selected for the development and maintenance of the relationship between *B. neritina* and calcium. The larvae of *B. neritina* are chemically defended by symbionts called *Endobugula sertula*, which are produced by bryostatins.

Biochemical and neurological research on bryostatins could lay the foundation for future investigations into their antipredatory effects (Paul et al. 2007). It is possible that symbionts produce defensive compounds other than bryostatins (McGovern and Hellberg 2003). It is not known which environmental factor is important for chemical defence and for the variable presence of a symbiont. It is possible that bryostatins give competition advantage to *B. neritina* because it is a relatively dominant and cosmopolitan species.

Bryostatins probably have an antipredatory effect in the snail *Polycera atra*. Bryostatins protect the conspicuous eggs of this species, which are laid into colonies of *B. neritina* (Paul et al. 2007).

Marine sponges have high nutritive value and lack physical defences. However, most sponges appear to be minimally affected by predators, competitors,

and other unfavourable organisms. This is possibly due to chemical antipredatory defences. Certain sponges are able to deter most fish. The sponges *Erylus formosus* and *Ectyoplasia ferox* contain triterpene glycosides that have antipredatory effects and other ecological functions (Kubanek et al. 2002). Some species or individuals employ not only chemical defences, but also rely on other strategies. Nevertheless, some extracts from the orders Hadromerida, Poecilosclerida and Haplosclerida are palatable for fish. The presence of symbiotic organisms in the tissues of otherwise palatable sponges probably has no distinctive effects. No relationship was found between the toxicity of sponge extracts and their ability to defend themselves from predators (Pawlik et al. 1995).

Furanosesterterpene tetronic acids (FTAs) deter fish of the genus *Ircinia* (Pawlik et al. 2002). Some species from order Hadromerida (e.g., *Cliona aprica, C. caribbaea, C. delitrix*, and *C. tenuis*) have also antipredatory effects (Chaves-Fonnegra et al. 2005).

Isocyano terpenes from sponges of the order Halichondriidae probably have anti-predatory effects too. Those compounds were found in other organisms as well, including bacteria, fungi, cyanobacteria and nudibranchs. Some nudibranchs probably take isocyano terpenes from certain sponges which they feed on, e.g. *Phyllidiella granulatus*, *Ph. pustulosa*. The true origin of isocyano terpenes and how the sponges prevent autotoxicity is not known. Cyanide is a potential toxin inhibiting respiration. It is possible (but this is untested) that sponges from the order Halichondrida contain yet further compounds with antipredatory effects (Paul et al. 2007).

Another chemical substance which repels predators is homarin. This substance is mainly found in snails of the suborder Cladobranchia (Affeld et al. 2007). The snail *Elysia rufescens* probably uses the compound kahalalide F as a chemical defence against predators (Molinski et al. 2009). Crude extracts from the above mentioned *Phyllidiella pustulosa* and also *P. varicosa* and *P. elegans* reduced fish feeding in Guam at natural concentrations. *P. pustolusa* occurs in both Guam and Palau, but only crude extracts from the Palau population deterred fish predators (Paul et al. 2007).

3. Bryostatins and other substances from bryozoans

Some marine bryozoans can be a rich source of pharmacologically promising substances. The most bioactive compounds isolated from bryozoans are the alkaloids (Prinsep et al. 2004). The most interesting metabolites are macrolide lactones (bryostatins), indole alkaloids (often bromine derivatives), isoquinoline quinones, sterols (steroids) and other carbohydrates with a heteroatom: nitrophenols, disulfides. The nitrophenols, bryostatins and some other metabolites are produced by bacterial symbionts. Establishing the activity of these compounds mainly depends on empirical tests. The antibacterial activity and/or cytotoxicity are the most significant effects. In several cases the metabolites have caused dermatic allergy, have shown antihelmintic activity or prevented cell division (Sharp et al. 2007). Bryostatin was discovered in marine bryozoans *Bugula neritina* (Davidson et al. 2001; Manning et al. 2005; Schmidt 2005; Sun and Alkon 2006; Mayer and Gustafson 2008). The bryozoan *Bugula neritina* has been investigated mostly in this regard (Davidson and Haygood 1999; Dahms et al. 2007).

Commensal microorganisms (e.g. bacteria, cyanobacteria, algae) which live in host organisms (including bryozoans) produce a large amount of bioactive substances. Bacterial origin has been confirmed in a very few cases, because bacterial symbionts occur mostly in microbial form (Paul et al. 2007). Bryostatins are an important group of pharmaceutically promising substances (Dunlap et al. 2007) produced by bacterial symbionts (Lopanik et al. 2006). It was long supposed that the sources of bryostatins are symbionts from bryozoans (Paul et al. 2007).

Bryostatins are produced by the bacterial symbiont *Candidatus Endobugula sertula*, which is present throughout all life stages of *B. neritina* (Lopanik et al. 2004b). The bryostatins are produced mainly by the mobile, short-lived larvae and early-stage juveniles (Lopanik et al. 2006). High concentrations of bryostatins were found in the tissues of zooids near the distal tips of branches (Winston and Woollacott 2008).

The study of Sharp et al. (2007) suggests that the bryostatins are deposited onto the exterior of *B. neritina* larvae during embryonic development, persist on the larval surface throughout metamorphosis and disappear prior to cuticle formation. During metamorphosis, '*E. sertula*' remains adhered to the larval pallial epithelium and is incorporated into the preancestrula cystid tissue layer, which ultimately develops into a bud and gives rise to the next zooid in the colony. Colocalisation of the bryostatin signal with aggregates of '*E. sertula*' in buds of ancestrulae suggested that they are syn-

thesised anew at these sites. In adult *B. neritina* colonies, symbiont microcolonies were observed in the funicular cords of rhizoids, which likely result in asexual transmission of '*E. sertula*' to regenerate colonies. Furthermore, the bryostatin signal was detected on the surface of the rhizoids of adult *B. neritina* colonies.

Although the cosmopolitan marine bryozoan *Bugula neritina* is recognised as a single species, natural products from this bryozoan vary among populations. Different populations contain different bryostatins (Davidson and Haygood 1999). At least three cryptic species of *Bugula neritina* live in the USA, and each contains different bryostatins (Paul et al. 2007).

On the basis of previous studies two chemotypes were defined: chemotype O contains bryostatins with an octa-2, 4-dienoate substituent (including bryostatin 1), as well as other bryostatins; chemotype M lacks bryostatins with the octa-2, 4-dienoate substituent. The results of genetic analyses suggest that the chemotypes represent different species.

Each species contains a distinct strain of *E. sertula* that differs at four nucleotide sites in the gene for the small subunit of ribosomal RNA (SSU rRNA). The results of the study of Davidson and Haygood (1999) indicate that the chemotypes have a genetic basis rather than an environmental cause. Both chemotypes are separated according to depth and warmth of water in the environment. Both produce bryostatins, but only the chemotype from deep and cold water produces bryostatin 1 (Mendla 2003).

Some northern forms of *B. neritina* do not have bacteria which can deter predators and their larvae do not contain bryostatins (McGovern and Hellberg 2003).

Bryostatins were also discovered in the snail *Polycera atra* from the group Nudibranchia. These animals feed on *Bugula neritina* on the surface of colonies and lay their eggs there. They perhaps take bryostatins with other bioactive compounds from the diet (Paul et al. 2007). Substances with bryostatin structure were discovered also in other marine organisms. Thus, *Bugula neritina* is not the only source of bryostatins (Manning et al. 2005).

Other medicinal effects of the bryostatins on the CNS of mammals have been discovered and researched (Paul et al. 2007). For example, it was found that bryostatin influences memory in the mollusc *Hermissenda crassicornis*, specifically, that bryostatin in a concentration of 0.1 to 0.5 ng/ml significantly enhanced memory. Concentrations higher than 1.0 ng/ml had a negative effect (Kuzirian et al. 2006). Bryostatin can also be used for the treatment of (Mehla et al. 2010), and prevention of HIV-1 infection (Ariza et al. 2011).

At least 20 different structures of bryostatin exist (Manning et al. 2006; Sun and Alkon 2006; Hale and Manaviazar 2010). The most frequently investigated substance from the bryostatin group is bryostatin 1 (Zhu et al. 2010). Bryostatin 1 is a potential drug for the treatment of leukaemia, lymphomas, melanomas, solid tumours (Davidson et al. 2001; Davidson and Haygood 1999), traumatic brain injury (Zohar et al. 2011) depression, Alzheimer's disease and other CNS disorders (Sun and Alkon 2006; Paul et al. 2007) and can activate innate immunity (Ariza et al. 2011). However, it has also been observed that bryostatin 1 causes intense muscle hyperalgesia (Alvarez et al. 2011). The other structures of bryostatin also have the potential to become important drugs (Davidson et al. 2001). The complete synthesis of some bryostatin structures has now been described, most recently, bryostatin 1 (Manaviazar and Hale 2010; Keck et al. 2011), 20-deoxybryostatin (Green et al. 2011), bryostatin 9 (Wender and Schrier 2011) and bryostatin 7 (Lu et al. 2011). Synthetic approaches to generating the bottom (Nakagawa-Goto and Crimmins 2011a) and top fragment of bryostatin 11 have also been described (Nagakawa-Goto and Crimmins 2011b). The synthesis of bryostatin analouges (bryologs) has also been reported (Wender and Reuber 2011; Wach and Gademann 2012). These are simpler and therefore lend themselves more easily to synthesis (Wender et al. 2011). A prime example is Merle 23, a derivative of bryostatin 1, differing from it only at four positions. It behaves like a phorbol ester in U-937 human leukaemia cells, while bryostatin 1 antagonises many phorbol ester responses in cells (Kedei et al. 2011).

The study of Davidson et al. (2001) demonstrates that it may be possible to clone bryostatin genes from *B. neritina* directly and use these to produce bryostatins in heterogeneous host bacteria.

Alkaloids from *Pterocella vesiculosa* have been also researched, with particular focus on their bioactivity, their cytotoxicity and antibacterial activity. Steroids from *Biflustra grandicella* have been studies as well. An extract from *Pterocella vesiculosa* possessed activity against P388 murine leukaemia cells. Two alkaloids, pterocellins A and B have been isolated from *Pterocella vesiculosa*. The biological activity of those alkaloids was determined, including their activities in an *in vitro* 60 cell line panel and *in*

vivo hollow fibre. The isolation and characterisation of further pterocellin analogues is currently in progress and tentative structures for two new members of this series, pterocellins C and D have been proposed, based on NMR and mass spectral data. Some alkaloids have been isolated from *Cribricellina cribraria*. All had antimicrobial and antifungal properties but in different degrees. Piramides A and F were isolated from *Amathia wilsoni*. Amathaspiramides A has mild antibacterial activity against *Bacillus subtilis* (Prinsep et al. 2004).

4. Bioactive substances from marine sponges

Marine sponges are a potential source of pharmacologically useful substances from marine organisms as they contain antibacterial, antiviral, antifungal and antiparasitic substances. More than 5 300 different products from marine sponges and their associated micro-organisms have now been described and more than 200 new metabolites from these organisms are reported each year. Some substances have the potential for clinically effective treatments. These include, e.g., ara-A (vidarabine), an anti-viral drug used against the herpes simplex encephalitis virus; manzamine, with activity against malaria, tuberculosis, HIV; lasonolides, with antifungal activity; psammaplin A, with antibacterial activity (Laport et al. 2005); spongistatins, with anticancer effects; mycalamides A and B, which inhibit protein synthesis causing apoptosis; pateamines, which harbour immunosuppressive and apoptotic properties; peloruside, with potential antibiotic activity; manolide (Dunlap et al. 2007), and luffariellolide (Ebada et al. 2010), with anti-inflammatory effects; chondropsin-class, which inhibited the growth of tumours (Dunlap et al. 2007); and halichondrin (Molinski et al. 2009), and geodiamolides A, B, H (Mayer and Gustafson 2008), with antiproliferative activity. The natural products spongouridine and spongothymidine from *Cryptotheca*, served as a template for the synthesis of the antiviral drug Ara-A (Vidaribine) that led to the development of acyclovir, which has effects against herpes virus and is used as medicinal drug for AIDS (azidothymidine). Later, Ara-A and spongouridine were discovered as natural metabolites from the Mediterranean gorgonian *Euniicella cavolinin* (Dunlap et al. 2007). Cytarabine from *Cryptotheca crypta* is now routinely used in the treatment of leukaemia and lymphoma. Its fluorinated derivative, gemcitabine has been approved for use in patients with pancreatic, breast, bladder and non-small-cell lung cancer (Md et al. 2012). Discodermolide was isolated from rare deep-water sponges, which are found only in the Bahamas. Discodermolide has immunosuppressive effects. However, trials using this substance were discontinued due to lack of efficacy and toxicity problems. The potential remains for its use in combination drug therapy. The sponge *Acanthella cavernosa* contains isocyano terpenes kalihinol A and kalihinol F, which inhibit the growth of the bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*. The growth of *Bacillus subtilis* and *Staphylococcus aureus* is inhibited by 3-formamido-1(10)-cadinene from the Paulan sponge *Axinyssa aplysinoides*. The isocyno terpenes kalihine A and B from *Acanthella klethra* have antifungal effects against *Mortierella ramannianus* and *Pennicillium chrysogenum* (Paul et al. 2007). Manoalide from the Palauan sponge *Luffariella variabilis* has antibiotic effects against *Streptomyces pyogenes* and *Staphylococcus aureus* (Ebada et al. 2010).

It is difficult to cultivate sponges and their microbial fauna. Valuable compounds must be extracted and purified from samples. The samples are collected by hand using scuba diving or with the aid of submersibles equipped with robotic arms. The awkwardness of both these methods complicates their use in the modern pharmaceutical industry (Molinski et al. 2009).

Much research in the field of natural products has focused on sponges. Only a small number of studies have focused on bryozoans for the following reasons: problems with accessibility, a lack of biomass available for extraction and difficult taxonomy (Pettit et al. 1993).

A summary of fungal and other symbionts in marine invertebrates as potential producers of biologically active (anti-infective, antiparasitic, anti-inflammatory, cytotoxic and other) substances is provided by the review of Debbab et al. (2011).

5. Bioactive substances from other organisms

Tunicates are also an important source of potential drugs. The peptide aplidine from *Aplidium albicans* has potential effects against acute lymphocytic leukaemia. A further compound, Ecteinascidin 743 (ET 743) from *Ecteinascidia turbinate* was approved as a drug (the trade name Yondelis) against

refractory soft-tissue sarcomas. ET 743 has important anti-tumour activity (Ebada et al. 2010) against solid tumours, in particular breast cancer and renal carcinomas, and soft-tissue sarcomas (particularly osteosarcomas, mesotheliomas, leiomyosarcomas and liposarcomas; Molinski et al. 2009). Species from the family Didemnidae contain different bioactive compounds, which have cytotoxic effects against fibroblasts and tumour cell lines (Dunlap et al. 2007). Didemnin B has possible efficacy for the treatment of pancreatic cancer in animal models (Mayer and Gustafson 2008).

Potential medicinal compounds have also been discovered in other marine invertebrates, for example dolastin from gastropod *Dolabella auricularia* (Dunlap et al. 2007; Mayer and Gustafson 2008). Phase II clinical studies which have tested the potential of dolastin as a cure for soft tissue sarcoma, melanoma prostrate and non-small cell lung cancers have been completed. The source of dolastin is probably cyanobacteria, coming from food (Dunlap et al. 2007). The first marine drug (Prialt) was approved in the United States in 2004 for the treatment of chronic pain in spinal cord injury. The source of Prialt is marine snails of the genus *Conus* (Molinski et al. 2009). Other compounds from this snail (conotoxins) have the potential to be highly effective drug candidates too (Park 2011). Another marine snail, *Elysia rufescens*, contains a compound called kahalide. Kahalide is currently in Phase II clinical trials (Molinski et al. 2009; Ebada et al. 2010) against solid tumours including melanomas, NSClC and hepatocellular carcinomas. The true source of kahalide is the alga Bryopsis, which the snail *Elysia rufescens* feeds on. *E. rufescens* accumulate high concentrations of kahalide. Kahalide content in *E. rufescens* is 1%, while its content in algae Bryopsis is 0.0002% (Molinski et al. 2009). Important anti-microbial effects were also discovered in marine snails. An extract from *Cenchritis muricatu*s was capable of completely inhibiting the development of *Staphylococcus aureus* and inhibited the growth of *Escherichia coli* by 95.9% (Lopez-Abarrategui et al. 2012).

An anti-inflammatory steroid was derived from the coral *Eunicea fusca*. However, its real source is a dinoflagellate (Dunlap et al. 2007).

The compound cephalostatin 1 from *Cephalodiscus gilchristi* (phyllum hemichordate) has potential effects for the treatment of chemoresistant tumours with potential defects in the mitochondrial pathway (Mayer and Gustafson 2008).

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