SLIME AND SCUM: NATURAL PRODUCTS FROM LAND AND SEA

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INTRODUCTION – WHAT ARE NATURAL PRODUCTS?

To a natural-products chemist, the term "natural products" does not refer to all compounds from natural sources, as the name might imply. It is specifically used to refer to compounds known as ***secondary metabolites***, structurally complex molecules, often of unknown function, with very limited biological distribution.

The primary aim of our research group is to isolate ***bioactive*** natural products. A bioactive (or biologically active) chemical has an effect on a living system, which may be good or bad. We screen these compounds to find useful molecules, for example, ones that kill fungi or tumour cells. Such compounds are potential new pharmaceuticals and can be used as research tools that help us understand the complex processes that occur in living organisms. We are also interested in compounds with novel chemical structures. Ideally, a new secondary metabolite will have both interesting activity and a novel structure.

Slime and ***scum*** are the types of organisms that we investigate to isolate ***second metabolites***. Slime refers to terrestrial ***cyanobacteria*** and scum to marine ***bryozoans***. W study these organisms in particular? Tests or screens on many different organisms over years have shown that they contain many bioactive compounds, which seem to help organisms in their everyday survival. Cyanobacteria, for example, live in a complex sc containing many other creatures, so need to compete for nutrients and space. It advantageous for them to produce compounds that may inhibit the growth of compet

* Dr Michèle Prinsep is a senior lecturer in Chemistry at the University of Waikato, and winner of the Zonta Science Award 2000. The award is administered by the Zonta Club of Wellington and has been offered biennially since 1990. Its aims are to encourage women to pursue a scientific career, to actively promote science as a career for young women, to encourage others already in the scientific field and to acknowledge the valuable contribution of women scientists. The prize consists of return travel to Britain, $5000.00 and a medal, which is presented to the recipient by the Governor General at a special reception held at Government House.

bacteria or fungi, leaving more resources for themselves. Marine bryozoans could also use chemicals to help them in their lives: they are primitive animals that are anchored to the seabed and are therefore, vulnerable to predation, overgrowth or infection. With no immune system, the theory is that bryozoans produce chemicals to help in their defence.

GENERAL APPROACH TO RESEARCH

How do we isolate bioactive compounds? The same process applies for both cyanobacteria and bryozoans. We extract the organism by homogenising it (mashing it up) in a blender with a suitable solvent. The extract is then filtered and the excess solvent is removed to leave a crude extract containing many different compounds, including the one that we are trying to isolate. Murphy's law means that this substance is often present in trace amounts only, so getting it out is a challenge. Fractionation techniques and various forms of chromatography are used to separate and isolate the compounds of interest.

Bioassays are tests that we use to monitor the isolation process and to determine in which fractions biologically active compounds are located. The assays that we use routinely screen for anti-tumour, anti-viral and anti-microbial activity, but we have access to some more specialised assays if required. Active fractions are combined and the process of fractionation and assay is repeated until, hopefully, we isolate a pure, active compound.

Once an interesting compound is isolated, the next step is to determine its structure. The most useful structural determination technique for the natural-products chemist is nuclear magnetic resonance (NMR) spectroscopy. This technique allows us to exploit the magnetic properties of certain atomic nuclei to determine molecular structure. Not all elements are observable by NMR spectroscopy but, luckily for the organic chemist, hydrogen and carbon are, so we can usually obtain a lot of structural information about organic compounds. Other techniques, such as mass spectrometry and X-ray crystallography, are also used.

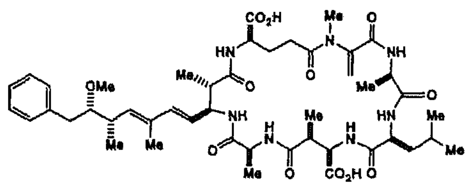
SLIME

What are cyanobacteria? They are neither plants nor animals. They look just like slime and are similar to bacteria in that they are usually unicellular and lack a distinct nucleus. However, unlike most bacteria, they contain chlorophyll and, like plants, carry out photosynthesis. A commonly used name for cyanobacteria is blue-green algae, even though they are not really algae at all and only some of them have the distinctive blue-green (cyan) colouration.

Why are we interested in cyanobacteria? They have existed for four billion years and are well adapted to their environment. They occur in a wide range of habitats such as fresh water, soil and salt water, from the cold of Antarctica to the heat of volcanic areas, so are very versatile organisms.

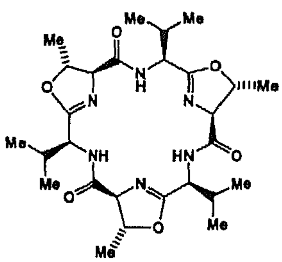
It is not feasible to collect sufficient quantities of cyanobacteria in the field for research into the secondary metabolites that they contain. Even if there is an 'algal bloom' in a water body, it will not be a clean, homogeneous culture. The solution to this problem is to collect a small sample of material, isolate an individual species and culture it in the laboratory under controlled conditions to produce enough organisms to work with. The cyanobacteria are cultured on a medium of inorganic mineral salts and provided with constant light and a low level of carbon dioxide as a carbon source. They are initially cultured in conical flasks, then transferred to larger vessels. Cyanobacteria have proven to be an excellent source of novel, biologically active compounds as the following examples illustrate.

Many different classes of compounds have been isolated from cyanobacteria, but the types of compounds most commonly isolated are cyclic peptides. Some of these, such as the ***microcystins***,[1-2] have proven to be a problem in lakes and rivers, causing poisoning and even death of stock which drink there. These compounds are hepatotoxins, damaging the liver of affected animals.



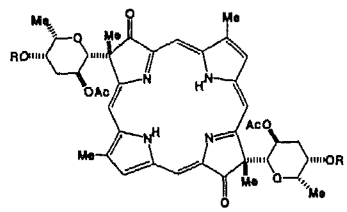
Microcystin-LA, one of the family of microcystins isolated from various species of cyanobacteria.

Other cyclic peptides isolated from cyanobacteria have beneficial properties. ***Westiellamide*** is a cyclic hexapeptide (containing six amino acids) that displays anti-tumour activity.[3] It was isolated from a terrestrial cyanobacterium from Hawaii. The same compound has been found in a marine organism called a tunicate, but in this case it is thought to be produced by the prokaryotic symbionts located in the tunicate cells.[4]



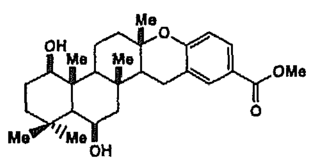
Westiellamide

Tolyporphin A was isolated from a cyanobacterium from Pohnpei in Micronesia.[5] It displays ***multi-drug resistance*** (MDR) reversing activity. Patients on prolonged chemotherapy become resistant to a wide range of therapeutic drugs. The cancer cells may actively pump drugs out, causing the resistance. Tolyporphin A reverses that resistance by binding to the cellular pump and inhibiting it, so that drugs can get into cancer cells to kill them.[6] We have subsequently isolated Tolyporphins B – K,[7-8] and conducted various studies on them, such as mode of action and metal binding studies. Tolyporphin A itself makes tumours more sensitive to a type of cancer treatment called ***photodynamic therapy*** and is currently under further investigation for use for this purpose.[9]



Tolyporphin A

Tolypodiol was isolated from the same cyanobacterium as tolyporphin A.[10] It is also a novel compound with excellent ***anti-inflammatory activity*** and has gone on to further investigation in the United States to assess its potential as an ***anti-arthritis*** compound.



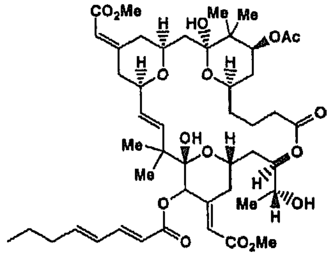
Tolypodiol

SCUM

Bryozoans or 'moss animals' are colonies of tiny filter-feeding individuals or ***zooids*** that are common fouling organisms on such things as ships, jetty piles and nets, hence the use of the term scum to describe them. Bryozoans are somewhat understudied biochemically, but work to date has indicated that they are an excellent source of novel, biologically active compounds.

The best example of this is the bryozoan species *Bugula neritina,* which is the source of a compound called ***bryostatin 1***.[11] This is currently in advanced clinical trials for a number of cancers, including ***melanoma*** and ***leukaemia***, and may well come on line as an anti-cancer drug.[12]

As stated above, bryozoans are understudied from a chemical point of view. There are several reasons why, including accessibility problems. Many bryozoans live below the depths that can be reached by divers and require the use of a dredge or submersible for collection. A submersible is very expensive and a dredge is very non-selective.



Bryostatin 1

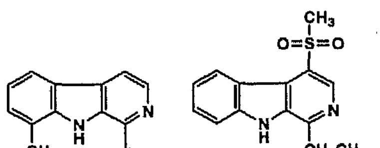
Obtaining sufficient quantities for research is another problem. Although some species are soft and feathery, most are instead heavily calcified. Therefore, very large quantities of material have to be collected to obtain enough to work with. Still other species grow in thin, encrusting forms that are also difficult to collect.

Accurate taxonomy is very important. You can get quite different colouration between different specimens of the same species, or you can have several samples that look identical but are in fact different species, which can only be distinguished by examination under the microscope. We are very fortunate to have expert assistance in bryozoan taxonomy from scientists at NIWA Oceanographic in Wellington.

Despite the problems associated with bryozoan research, New Zealand is quite a good place in which to study them. Obviously, we are surrounded by water and have a long coastline and we also have a very good species diversity of bryozoans with a high proportion of endemic species.

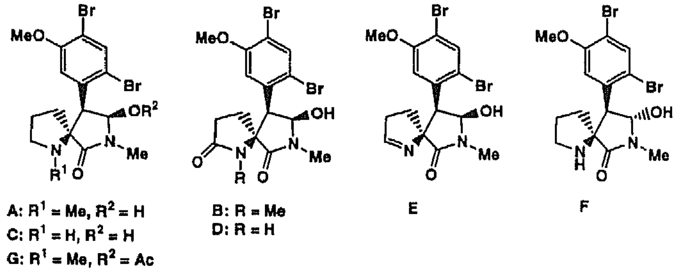
Our research strategy is to collect as wide a species diversity as possible, extract them and screen for biological activity.

Investigation of a New Zealand bryozoan, *Cribricellina cribraria* resulted in the isolation of a series of ***β*-carboline alkaloids** (of which two novel ones are shown below). These exhibit a range of biological activities including *anti-tumour* and *anti-fungal* activities.[13] Other bryozoans in the same family contain these alkaloids, but composition and quantities vary quite widely.[14]

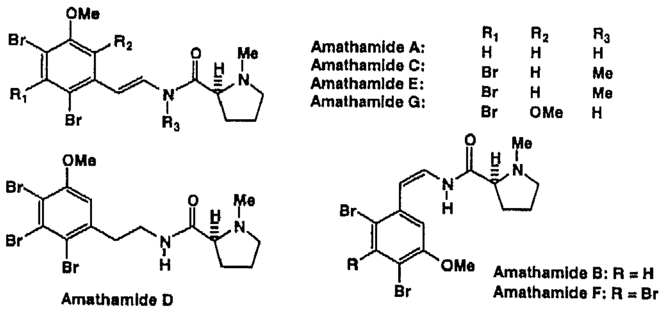


Novel alkaloids isolated from *Cribricellina cribraria*

Some recent examples of our work include an investigation of the bryozoan *Amathia wilsoni*, which contains a series of alkaloids, the ***amathaspiramides***.[15] These compounds are structurally very similar to a series of compounds called the ***amathamides***, which were isolated from Australian collections of *Amathia wilsoni.*[16-18] Interestingly, although both Australian and New Zealand organisms have been identified as the same species taxonomically, the Australian samples only seem to produce amathamides, while New Zealand colonies only produce the amathaspiramides.

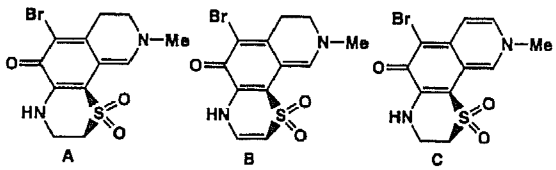


Amathaspiramides A-G



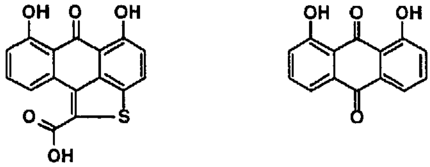
Amathamides A-G

The species *Euthyroides episcopalis* contains the ***euthyroideones,*** which represent examples of a new ring structure.[19] These compounds contain an unusual combination of functional groups and are very interesting from a structural point of view.



Euthyroidenes A-C

The bryozoan *Watersipora subtorquata* contains some compounds described by their basic ring structure as ***anthraquinones***. This ring structure was already known, but our group was able to carry out research into their biological activity and determine how altering various functional groups in the structures affected the biological activity.[20]



Anthraquinones isolated from *Watersipora subtorquata*

This ongoing research demonstrates the utility of natural products research as a source of compounds that may be useful as pharmaceuticals and/or are of structural interest to a chemist. As we search for potential new pharmaceutical compounds in both terrestrial cyanobacteria and marine bryozoans, we are constantly amazed at nature's ability for organic synthesis.

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