Chapter 32

Natural substances: hidden treasures

Sylvain Petek

Gorgonians and alcyonaria are among the organisms tested for biological activities. © IRD/S. Andréfouët

For most people, "natural substances" are closely associated with traditional medicines, ethnopharmacology, herbal medicine and even aromatherapy. This association of ideas comes from multi-century-old knowledge of the uses, mainly of terrestrial plants, in curing various diseases in different communities around the world. Behind this notion, lies a more general interest in molecules synthesized by organisms, particularly those with specific biological and/or therapeutic activities.

Regardless of the organism under consideration, the molecules it produces are generally classified into two groups: those essential to life, known as primary metabolites (amino acids, nucleotide [ADN, RNA...], fatty acids with membrane function, etc.) that are found in very different taxonomic groups of organisms; and those known as secondary which are not involved in fundamental physiological functions, and are generally specific to the type of organism considered (plants, bacteria, fungi, etc.). The latter will be the focus of the present chapter.

From chemical ecology to applications inspired by nature

During evolution, organisms have developed a whole range of secondary metabolites for adapting to the physical (luminosity, temperature, pressure, salinity, etc.) or biological (predation, colonization, infestation, etc.) variations of their environment, and to communicate. Transdisciplinary studies of chemical ecology, involving both chemists and biologists, make it possible to isolate, identify and understand the role of these compounds. Thanks to this work, innovative, nature-inspired and more environmentally friendly solutions can be developed, whether in the realms of human and animal health, agronomy, aquaculture or other technological sectors.

Medicines from the sea

Although the use of terrestrial plants is a very old practice and widespread throughout the world in the various pharmacopoeias, the historical use of marine organisms is mainly known from China and the Far East. The Chinese Pen Ts'ao, published 2,800 B.C., contains a chapter exclusively dedicated to the use of algae for the treatment of gastric ulcers or goiter, for example. Much more recently, the Japanese have used a red alga, called *kaïniso (Digenea simplex)*, containing kainic acid, as an anthelmintic (antiparasitic), which has led to the preparation of a proper drug against *Ascaris*. Outside this geographical area, there is hardly any oral tradition or traditional medicine referring to the use of marine organisms.

It was from the mid-20th century onwards, with the development of new tools for underwater exploration and analysis, and especially from the 1970s, that systematic studies of marine biodiversity for uses in human health really began.

Life appeared in the oceans and they therefore still harbor all existing life forms. Of the 33 main lineages, 12 are exclusively marine and others are essentially marine (sponges or cnidarians [jellyfish], etc. of which there are a few freshwater species) - in other words, there is a whole range of marine biodiversity that is unparalleled in terrestrial and freshwater environments. In addition, seawater contains chemicals such as halogens (chlorine, bromine, iodine, fluorine), sulfur and metals that are not readily available elsewhere. This biological diversity, combined with the chemical specificities of the marine environment and the first encouraging discoveries, gives rise to many hopes for the emergence of a new marine pharmacopoeia.

For instance, the first cephalosporins, a family of antibiotics widely used today, were discovered in Italy in 1948, with the cultivation of *Cephalosporium acremonium*, a microscopic fungus found in lagoon sediments. The family of Arabinosides, with anticancer and antiviral properties, was inspired by isolated compounds of a Caribbean sponge, *Cryptotethya crypta*, in the 1950s.

Faced with this extraordinary biodiversity and without ethnopharmacological knowledge to guide them, the task of researchers is immense. In an attempt to select the most promising organisms, in situ observation of their behavior can provide some information. For example, organisms without physical protection that are not suffering from epibiosis, predation or grazing are likely to have developed a chemical cocktail designed for their defense. To study these organisms, different approaches have been used. Some are rather "systematic" (Fig. 1), without preconceptions as to the biological activity of the organism and with bioassays being carried out on isolated molecules. Others use bioguidance (Fig. 2), which first involves the selection of organisms based on their activity on a biological target (bacteria, enzymes, cancer cells, etc.) and the progressive isolation of the active substance(s) responsible for the observed activity. Each approach has its advantages and disadvantages.

After more than 50 years of research, a true marine chemodiversity has been discovered, with about 29,600 molecules isolated to date, a large part of which has no terrestrial equivalent.

As shown in Figure 3, just over one-third of the compounds are derived from sponges and/or their associated microbiomes. These sessile animals (chap. 13), unable to escape their predators, have

developed a "chemical arsenal" for their defense, for colonizing new areas and protecting themselves against pathogens. In addition, sponges belong to a lineage whose compounds provide the widest spectrum of biological activities: antibiotic/antibacterial, antifungal, anticancer, anti-inflammatory, antiviral, antimalarial, immunostimulant, antispasmodic, etc.

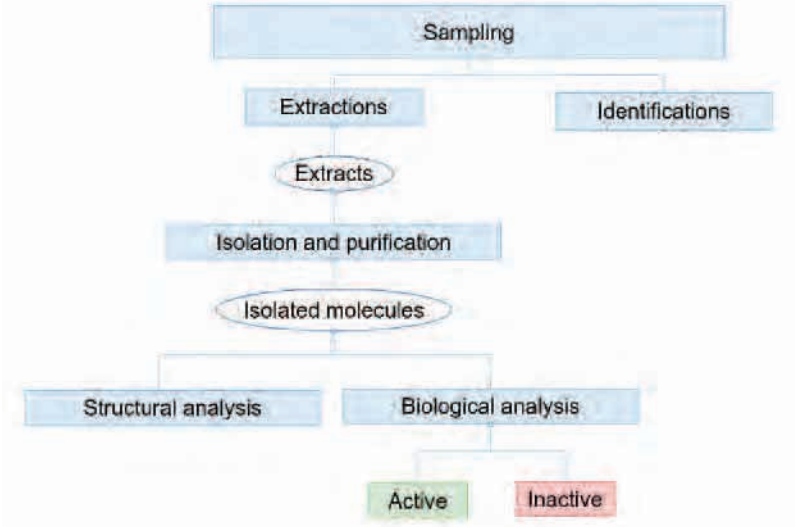

Figure 1: The "systematic" approach. © IRD/S. Petek

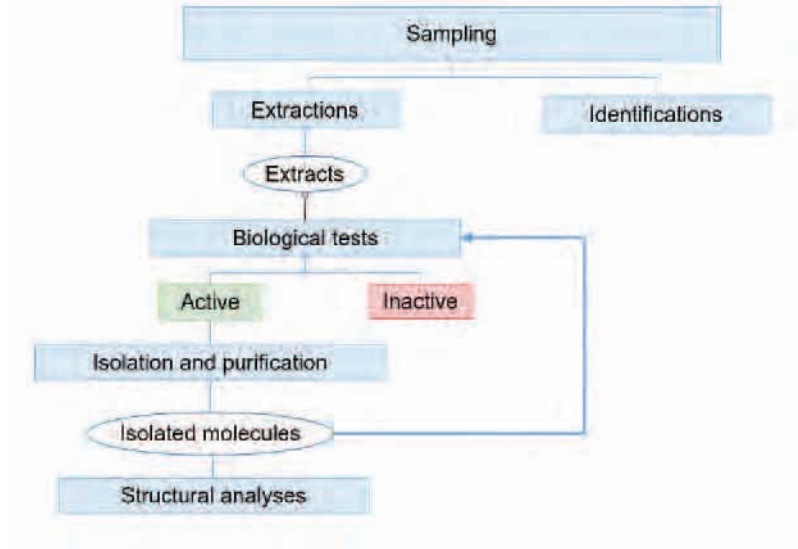

Figure 2: The "bioguided" approach. © IRD/S. Petek

Currently, in addition to cephalosporins (antibiotics, 5th generation since 1964), nine drugs of marine origin (or derivatives) have been placed on the market for anti-cancer, antiviral, analgesic and antiparasitic treatments. Some fifteen other molecules are being used in clinical trial assays. These figures may seem small in terms of the number of molecules discovered, but they are actually relatively significant. This is because, in pharmaceutical research, only one molecule out of every 10,000 will become a drug and it takes an average of 12 years between the discovery of the molecule and its launch on the market.

The valorization of marine natural substances often faces challenges related to resource access, availability and the environmental impact of industrial exploitation. Consequently, their use often involves the development of their synthesis by chemical and/or biotechnological methods or the production of simpler derivatives, where the aim is to keep only those fragments of the molecule necessary for the activity. This can also involve the addition of other functions which will provide complementary qualities in terms of assimilation, stability or target, known as pharmacomodulation. In this context, natural marine substances will be a source of inspiration for the discovery of original bio-active chemical structures rather than a resource as such.

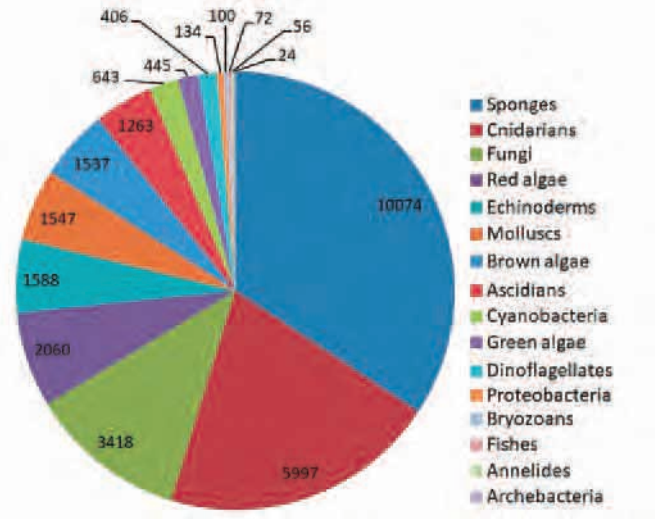

Figure 3: Distribution of molecules discovered by lineage. © IRD/S. Petek

Box 26

Cantharella, a database to capitalize natural substances

Sylvain Petek and Adrien Cheype

When we want to study natural substances[24], whatever their origin, we soon find ourselves confronted with managing a large volume of data of diverse natures and origins:
– sampling sites: country, locality, GPS point, species inventory, environmental/biotope information, etc.;
– taxonomic identification of the sampled organisms, their abundance, physical and genetic characteristics, etc.;
– chemical protocols implemented, molecules identified;
– biological activity assays performed.
In addition, these studies are multidisciplinary and require the support of many specialized collaborators, who are often geographically distant.

In the end, only part of this information will be included in scientific publications and thus permanently recorded. In the long run, therefore, there is a risk that the "raw" data may become unusable or disappear when it could provide historical records and serve as a basis for new projects. In addition, over time, the heterogeneity of paper or computer media, file formats, or the way data is structured make it very difficult to reuse information efficiently.

Cantharella (PETEK and CHEYPE, s.d.), a database dedicated to the study of natural substances has been designed to provide a solution to the various challenges arising from these data, in terms of:
– access and sharing between collaborators or transfer to collectivities;
– analysis and updating;
– long-term sustainability.

This collaborative tool, accessible online and developed from "free" software packages, uses four specialized modules to capitalize all the data from the field collection of organisms through biological assays to identified molecules.

In addition, as part of the Access and Benefit-sharing process (ABS, Nagoya Protocol), the tool provides a platform for the transfer of results to communities, who can thus monitor the research that is being done on their biodiversity. For universities or laboratories wishing to use it, the software is made available under a free license (https://forge.codelutin.com/projects/cantharella).

The IRD's instance of Cantharella, operational since 2010, is capitalizing on data from numerous projects, mainly in the Pacific (about 700 sampling sites and 950 species, and over 7,700 bioassay results).

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Explorations of New Caledonia's marine chemodiversity

In New Caledonia, the first marine bioprospecting studies targeting new molecules of therapeutic interest began in 1976 at ORSTOM with the research program Snom (Substances naturelles d'origine marine). This program was led by Pierre Potier (Institute of chemistry of natural substances, CNRS) and involved researchers and scientific divers from the IRD (ex-ORSTOM) and the taxonomic expertise of MNHN. Over the years, numerous other explorations, studies and research programs have been conducted, which involved several French and international multidisciplinary collaborations (Fig. 4).

A wide diversity of geographical zones, environments and habitats has been explored: from Grande Terre to the Loyalty Islands, the Isle of Pines, remote atolls and reefs (d'Entrecasteaux and Chesterfield), from lagoons to outer slopes of barrier reef, or seamounts (chap. 2).

Currently, out of all lineages, ranging from micro- to macro-organisms, biological analyses and/or activity assays have been carried out on a total of 9,372 species. Extensive pharmacochemical studies on about 50 organisms have isolated and identified more than 350 new bioactive molecules with original structures, including over 100 from sponges.

A few emblematic examples

Girolline, a tiny molecule extracted from *Cymbastella cantharella*, a sponge living on the outer slope of the southern barrier reef, was found to be particularly active during in vitro and in vivo assays on cancer and tumor cells. Without exhibiting major toxicity in mice and dogs, clinical studies were conducted up to phase II with the pharmaceutical company Rhône-Poulenc Rorer (now Sanofi-Aventis), before being interrupted due to side effects on the cardiovascular system. In addition, girolline has demonstrated interesting antiplasmodic activities *in vitro* on four strains of *Plasmodium falciparum*, particularly in synergy with chloroquine, paving the way for new antimalarial strategies.

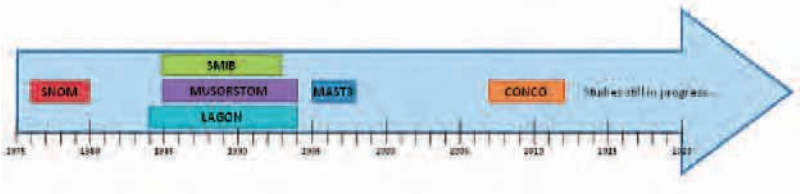
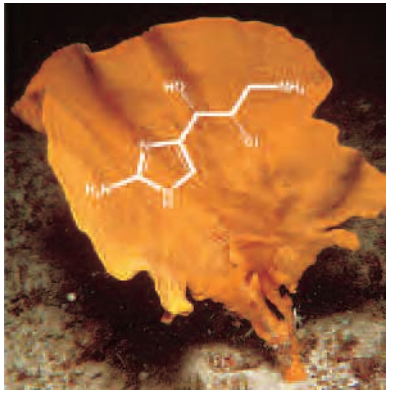
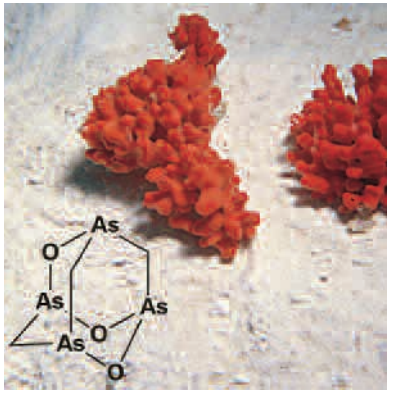


Figure 4: Main bioprospecting and therapeutically oriented research programs. © IRD/S. Petek

Laticauda Laticaudata and the erabutoxin b formula. © IRD/P. Laboute


The sponge *Cymbastella cantharella* and the girolline formula. © IRD/J.-L. Menou



The sponge *Echinochalina bargibanti* and the arsenicin A formula. © IRD/G.Bargibant

Arsenicin A, produced by *Echinochalina bargibanti*, a sponge from the eastern lagoon of Grande Terre, is distinguished by its nested polycyclic formula with four arsenic atoms, which is very unusual for an organic molecule of natural origin. It has bactericidal, fungicidal and antiproliferative properties on acute promyelocytic leukemia cells, as well as on pancreatic adenocarcinomas and glioblastomas.

The crinoid *Gymnocrinus richeri* and the gymnochrome B formula. © IRD/P. Laboute

The crinoid *Gymnocrinus richeri*, an echinoderm considered living fossil and sampled at a depth of 520 m on the Norfolk Ridge, led to the discovery of a new family of pigments: the gymnochromes, which have antiviral, anti-HIV and anti-dengue properties.

Poisonous cones, mollusks that paralyze their prey by injecting them with a mixture of neurotoxic peptides, are particularly promising for the discovery of powerful analgesics, such as Prialt® (1,000 times more powerful than morphine). A full research program is dedicated to the study of the genome and venom composition of *Conus consors* from the Chesterfield Reefs.

Lastly, the very emblematic sea kraits, *Laticauda colubrina* and *Laticauda laticaudata*, belonging to the same family as cobras or mambas (Elapidae) produce a particularly potent venom of which the polypeptide erabutoxin b, one of its main components, has been studied for its effects on the neurological system.

After all these years, only part of the pharmacochemical potential of the marine biodiversity of New Caledonia has been extensively studied (sponges, cnidarians, ascidians, etc.). The potential bioactive molecules of other biological groups and species have yet to be explored, rediscovered or valorized using recently developed biological and chemical techniques. Biotechnological developments involving micro-organisms are very promising in various scientific domains, such as microalgae for the production of biofuels or high added-value compounds for cosmetics or nutraceuticals.

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