

MARINE LIFE SCIENCE

The fascination of interdisciplinary scientific disciplines such as marine chemistry, microbiology and chemical ecology draws from a huge variety of new structures, biological activities, and highly effective substances, which have evolved during millions of years of evolution in marine food and communication chains. Seventy percent of the earth's surface is covered with water. All life has begun in the ocean and has proceeded from there to an enormous species richness. Marine macroorganisms (sponges, tunicates, soft corals) and microorganisms (bacteria, fungi, unicellular *algae*) provide an almost inexhaustible reservoir of natural compounds which can principally be exploited for technical and medical applications. While early marine research focussed on tropical regions, today's investigations include temperate and even polar regions. Scientists are still beginning to discover the potential of the ocean.

Coral Reef Ecosystems

Coral reefs ecosystems are populated by organisms that thrive in close relationship with each other. In that respect, coral reefs are comparable to tropical rainforests, where space and nutrients are scarce. Thus, the need to adapt to these special living conditions resulted in an extraordinary diversity of plants, animals, and microorganisms. Many reef inhabiting animals live in *symbiosis* with microorganisms. For example, corals are hosts to symbiotic microalgae (zooxanthellae), which provide them with important nutrients. Marine *sponges* may also contain large amounts of microorganisms, which can account for almost 40% of the sponge's biomass. Yet little is known about the function of these microbes. Inhabitants of coral reefs permanently compete for space and have to find strategies to avoid being overgrown by biofilm-forming microorganisms (biofouling). Additionally, many marine invertebrates are permanently attached to a surface (termed "sessile") and do not have a shell, teeth or claws for defense. Therefore many strategies have evolved in the coral reef ecosystem to secure space and to optimize chances of survival. These strategies include the excretion of toxic compounds or of compounds that are inhibitory to the growth of others. Also, some organisms might out-space others simply by overgrowing them. This "fight for survival" is frequently based on *natural products* with potential for phar-



The dense population of coral reefs and the resulting competition among organisms for space and nutrients is probably the reason for the chemical defenses developed by many sessile reef organisms.

macological, medical or (bio) technological applications.

The development of marine natural products as drugs is a challenging endeavor. Many potentially interesting substances can only be found in minimal amounts and therefore the collection of enormous biomass would be needed for a sufficient supply of the substances. Overexploitation of marine reefs is prohibited. There are, however, alternative strategies to secure the supply of natural products in a sustainable manner, such as total or partial chemical synthesis, marine *aquaculture* and growth in controlled laboratories. Additionally, *molecular genetics* tools are used to provide distinct marine natural products. Successful cloning of *biosynthetic gene* clusters into cultureable organisms would be of enormous help to supply larger amounts of substances.

Aquaculture
Culture of aquatic organisms.

Algae
Uni- or multicellular organisms which are capable of photosynthesis but do not belong to the plants. Algae consist of protists, diatoms, and red-, green- and blue algae.

Symbiosis, symbiotic
Different organisms living in and profiting from the close relationship. For example, unicellular photosynthetic algae living in corals and securing the survival of their hosts in low nutrient, tropical waters.

Sponges (Porifera)
A deeply branching and evolutionary ancient lineage of the animal kingdom. Early developmental stage of multicellular organisms.

Natural product
Biogenic substance which occurs in some but not in all organisms (secondary metabolite).

Molecular genetic
Genetics based on nucleic acids (DNA and RNA)

Biosynthesis gene
Responsible for the synthesis of secondary metabolites

Alkaloids

Basic secondary metabolites, mainly found in plants, which contain mostly one or more heterocyclic attached nitrogen atoms

Total synthesis

Field of organic chemistry, which aims the most efficient assembly of secondary metabolites starting from abundant precursors

Pyrrol imidazol alkaloids

A group of basic natural products

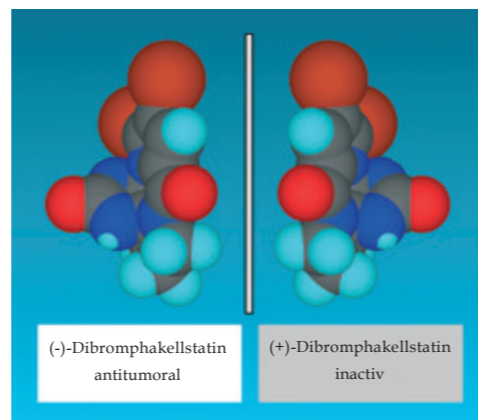
The Caribbean sponge *Agelas conifera*, a source of the pyrrole imidazole alkaloids.

Marine Secondary Metabolites – Surprising Structures and Exceptional Properties

Natural product chemists and biologists are an enthusiastic bunch and marine chemistry with its exceptional molecules and unknown reactivities and functions maintains a great fascination to them. For example, the cytostatic and immunosuppressive substance palau'amine, extracted from the marine sponge *Stylorella aurantium*, displays an internationally intensively studied – in 2010, the first total synthesis of racemic palau'amine has been accomplished – aim of *total synthesis* and belongs to the exclusively marine group of *pyrrole imidazole alkaloids*.

Another example is the tetracyclic alkaloid dibromophakellstatin, which was isolated from the marine sponge *Phakellia mauritiana* in 1997 and showed cytostatic activities in first trials. Further research on dibromophakellstatin was hampered by the lack of sufficient material: 170 kg of sponge tissue were needed at that time to isolate 31 milligrams of secondary metabolite. To further address the question whether dibromophakellstatin can become important to marine life sciences, it was necessary to establish new and independent ways of accessing it by total synthesis.

In this context, chemists become architects. It is essential to assemble a complicated secondary metabolite within as few steps as



Enantiomers of dibromophakellstatin with different biological properties

possible in optimized yield. A perfect total synthesis would be, if commonly available compounds react in a single step forming the required product. But researchers are far away from that. In reality, the total synthesis of dibromophakellstatin currently needs 8 subsequent steps. Now, at least, it is possible to obtain that secondary metabolite in a hundredfold amount when compared to isolation – a necessary step towards all further studies.

There is another problem, which can be observed with nearly every secondary metabolite. There exist two non-identical mirror images – like right hand and left hand – of which only one is biologically active. Interdisciplinary collaboration between chemistry and medicine discovered, that dibromophakellstatin has to show a new mode of action – a trigger for biochemical studies, which will focus on the research of interactions of dibromophakellstatin and proteins. Secondary metabolites and proteins belong together.

What about the biological functions? Marine secondary metabolites regulate the defence and communication of sessile living organisms. In the case of pyrrole imidazole alkaloids, the cooperation between organic synthesis and marine chemical ecology proved that the key metabolite oroidin functions as fish deterrent and assures the survival of the *Agelas* sponges within the reef community. The pharmacological fundamentals of this effect still remain unclear. Recently, it was discovered that pyrrole imidazole alkaloids also inhibit biofilm formation.

Another exciting field concerns the biosynthesis of marine natural products. Sponges

MARINE NATURAL PRODUCTS IN CLINICAL USE

SPONGONUCLEOSIDES

The ribose unit of nucleosides can be replaced by arabinose as is the case in the marine sponge *Tethya crypta*, which contains spongouridin and spongothymidin. Both lead structures, discovered as early as 1951, initiated the development of the antimetabolites cytarabine (ara-C) and vidarabine (ara-A), which are clinically applied for the treatment of myeloid leucemia and of viral infections, respectively.

PSEUDOPTEROSIN E

The glycosylated diterpenoid pseudopterosin E from the gorgonian *Pseudoptero-gorgia elisabethae* made its way into the skin cream Resilience®. The antiinflammatory properties of the pseudopterosins were discovered in 1986 by Fenical and Jacobs. They are caused by the inhibition of prostaglandin and leucotriene liberation by macrophages. The content of pseudopterosins in *P. elisabethae* can be extremely high, reaching 15 % of the dry weight. Thus, the natural product can be supplied by collection of its source organism.

ω-CONOTOXIN MVIIA

In 1984, Olivera isolated ω-conotoxin MVIIA (ziconotide, SNX-111, Prialt®) from the marine cone snail *Conus magus*. The 25 amino acid peptide became the first structurally unmodified marine natural product to reach a truly clinical application. In 2004 ω-conotoxin MVIIA was approved for the treatment of opioid resistant pain. Treatment requires less than 10 µg per day. However, the compound must be administered intrathecally by injection into the spinal fluid. ω-Conotoxin MVIIA selectively blocks N-type calcium ion channels in subnanomolar concentrations.

MARINE NATURAL PRODUCTS IN CLINICAL USE

ECTEINASCIDIN 743

The trisisoquinoline alkaloid ecteinascidin 743 (Trabectedin, Yondelis®) was discovered independently by the Rinehart (patent of 1988) and Wright groups who isolated the compound from the ascidian *Ecteinascidia turbinata*. Since 2007, ecteinascidin 743 has been approved as a drug for the treatment of soft tissue sarcoma. It has been established that ecteinascidin 743 binds to the minor groove of DNA. Supply of the natural product is secured by partial synthesis starting from the microbial metabolite cyanosafrafin B, which can be obtained by fermentation of the bacterium *Pseudomonas fluorescens*. The partial synthesis was adapted from an earlier total synthesis by the Corey group. There are also aquacultures of *E. turbinata*.

The tunicate *Ecteinascidia turbinata* is grown in aquaculture for the production of antitumor compounds.



comprise complex communities constituted by the invertebrate host and by associated microbial symbionts. It could be shown that the sponge cells themselves contained the pyrrol imidazole alkaloids. The sponge associated microorganisms on the other hand are devoid of these metabolites.

Moving on from biologically active alkaloids of a few amino acids in size to higher



The development of new glues profits by the bivalves' mechanism of natural adhesion to stones and rocks.

molecular weight substances derived from marine organisms, the green fluorescent protein (GFP) molecule is of outstanding importance, acknowledged by the Nobel Prize in Chemistry 2008. About forty years have passed since the original isolation of GFP from the jellyfish *Aequorea victoria*. Finally, molecular genetics enabled the determination of the complete amino acid sequence. After transfer of the *gene* into a bacterium, it was stimulated to produce high amounts of ultrapure GFP. Its three-dimensional protein structure was clarified in 1996 via X-ray diffraction analysis of single crystals. The outcome was also aesthetically appealing: the green fluorescent protein is a so-called "β-barrel", whose walls are assembled by eleven anti-parallel peptide chains belonging to a single strand. An α-helix with an incorporated fluorophore is situated inside the barrel like a wick in a candle. This chromophore renders GFP as indispensable tool of modern biochemistry. It is possible to combine the GFP gene with a gene of interest. Wherever that gene of interest will be expressed, there will be an additional expres-

sion of GFP which can be easily analysed under UV light due to its green fluorescence. Thus it is possible to trace down the viral infection of cells or to generate green fluorescent mice for medical applications. By now, several colours have become available.

Marine Toxins

Everyone who has had contact with jellyfish, corals or sea urchins during summer vacation, is painfully aware of the role *toxins* play in the ocean. Toxins serve various purposes ranging from defence and feeding deterrence to hunting prey. The most famous toxins are probably tetrodotoxin, produced by bacterial symbionts of the pufferfish, and saxitoxin, potentially occurring in mussels. Another example is ω-conotoxin MVIIa from the cone snail *Conus magus*, which has been available as a drug in the USA since 2004. Originally more than 100 peptide toxins were isolated from the toxin of conus snails, of which one was shown to disable the sensation of pain by blocking of sodium channels.

New Incentives for Material Sciences

Besides the development of new drugs and their use as tools in biochemistry and cell biology, marine natural products may also conquer the material sciences. Technical innovations can profit from the precise observations of nature: why are bivalves so closely attached to rocks and why are they not crushed by even the strongest waves? Why do many algae not succumb to biofouling by other organisms? These questions are begging for more detailed investigations and are standing at the onset for the development of new glues and ship coatings.

The red algae *Delisea pulchra* contains halogenated furanones in specialized cells which are slowly released at the surface of the plant and which effectively prevent biofouling of the algae by micro- and macroorganisms. An Australian company was subsequently founded which will attempt to incorporate the furanones into polymer plastics. The advantages of this anti-biofouling strategy are apparent. If the furanones can eventually replace the toxic tributylstannyl compounds that are currently used in ship and boat paints, then the toxicity problem would be much reduced.

MARINE NATURAL PRODUCTS IN CLINICAL USE

DEHYDRODIDEMNIN B

The ascidian *Trididemnum solidum* is the source of the didemnins, cyclic peptides consisting of unusual amino acids. In 1981, the Rinehart group reported on the cytotoxic activity of a few of those natural products which had already become apparent on board of a research vessel. Didemnin B was forwarded to clinical testing in 1986, but showed neuromuscular toxicity. However, an oxidized derivative of didemnin B, dehydrodidemnin B (aplidin®) finally became an orphan drug in 2004 for the treatment of myeloma and lymphoblastic leukemia.

KAHALALIDE F

Among the clinical candidates against prostate and lung cancer, there is the *depsipeptide* kahalalide F from the nudibranch *Elysia rufescens*. Interestingly, the diet of that nudibranch, the green alga *Bryopsis* sp. also contained kahalalide F. The absolute stereochemistry of kahalalide F was established by total synthesis in 2001. Kahalalide F obtained its name from the Kahala bight of the Hawaiian island of Oahu, in proximity to the institute of Paul Scheuer, the discoverer and founder of the field of marine natural products chemistry. Kahalalide F causes cell *necrosis*, but not *apoptosis*. Its cytotoxicity is about a 100-fold weaker than in the case of didemnin B, thus potentially opening a wider therapeutic window.

MARINE NATURAL PRODUCTS IN CLINICAL USE

ERIBULIN

Eribulin (E7389) is a partial structure of the sponge secondary metabolite halichondrin B, which has been isolated from *Halichondria okadai* by Hirata and Uemura in 1986. The Kishi group clarified the stereochemistry of halichondramide B by total synthesis. It turned out to be wise to study the biological activity of synthetic intermediates and smaller fragments of halichondrin B. Eribulin is clinically tested (phase III) as an antitumor compound against breast and prostate cancer.

SALINOSPORAMIDE

Salinosporamide carries hopes to become a clinically important marine natural product and is mentioned here as member of the pipeline of potential drugs. Salinosporamide was discovered by the Fenical group and stems from the exclusively marine bacterium *Salinispora* sp., which grows on sea water media. The compound is a covalent proteasome inhibitor and acts by a mechanism which is rarely seen among anticancer drugs.

Depsipeptide

A peptide with ester and amide linkages

Apoptosis

Cell death

Membranes

A lipid layer surrounding cells or nuclei

Necrosis

Death of cells and tissues

The soft coral *Pseudoterogorgia* sp. is the producer of antiinflammatory compounds that are being added to cosmetic products.





The caribbean sponge *Xestospongia muta* ("barrel sponge") contains large amounts of microbial symbionts which may be involved in secondary metabolite production.

Gene expression

Translation of gene encoded information into proteins.

Many beach combers have noticed the extraordinary strength with which mussels are attached to their substratum on rocky shores. Because of the toughness of the glue which is due to its polymeric properties, identifying the chemical nature of the mussel glue was a scientifically difficult problem. While spectroscopic data could be obtained, they did not suffice to solve the problem. Only the chemical synthesis of

lead compounds, whose physical properties were similar to those of mussel glue, helped understand the chemical structure of mussel glue. The reason for their strong bonding properties was found in the presence of modified proteins that contain Fe(III)-complexes.

An outstanding discovery on the border to inorganic chemistry concerns the involvement of the silaffins in the making of silica-containing shells by diatoms. Silaffin 1A1 is a small peptide with many phosphorylations and with polyamine side chains. This discovery enabled the production of silica gel-nanospheres of a controlled size.

The search for marine natural products functioning as catalysts would be a worthwhile endeavor. The few derivatives from natural products that are currently used were probably only discovered because they are abundant structures on the shelf.

Marine Drugs

Animals inhabiting coral reefs, such as sponges, tunicates, bryozoans and corals have been the most prolific source of natural products, yielding more than 20.000 compounds today. Natural products have been shaped through evolution towards maximum potency. They are more suitable when compared to organic structures that have been synthesized randomly. More than half of all substances in clinical trials over the last 20 years have been modelled after a biologically active natural compound.

Quite a few marine natural products have entered clinical trials, most of them as anticancer agents, followed by compounds against inflammatory diseases and pain that cannot be treated by morphine.

Several international research groups focus on the identification of novel anti-infectives from marine sponge-associated microorganisms. Following reports of the World Health Organisation (WHO), infectious diseases are still the number 1 of cause of death. With respect to the increasing appearance of multi-drug resistant pathogens, the search for novel antibiotics is an urgent endeavor. Of interest are antibacterial compounds and also those that interfere with the gene regulation and *gene expression* of virulence factors. A specific focus is placed on biofilm-inhibitory substances because biofilm-production by clinical Staphylococci results in the dysfunctioning of catheters and other medical devices.

There are also natural products that turned out to be clinically less than originally assumed. For bryostatin 1 and the dolastatins a clinical use as anticancer medication has become unrealistic. The prominent role of bryostatin 1 has nevertheless provided important momentum for the continued development of the scientific discipline "Marine Medicine". From a chemical perspective, the total synthesis of bryostatins has been accomplished. The technology evolving around aquaculture of marine invertebrates will support commercial development. The endosymbiont, *Candidatus Endobugula sertula*, which is symbiotically associated with the bryozoan *Bugula neritina* has been discovered and was shown to contain the polyketide synthase gene cluster which is probably responsible for the synthesis of bryostatin.

It would even be conceivable that bryostatin 1 will have a renaissance. Neurobiologists have become interested in this substance as it improved the cognitive properties of the snail *Hermissenda*. After four hour exposure to 0.25 ng/ml bryostatin, the memory of the snail was extended from seven minutes up to one week. A new career of bryostatin in Alzheimer-Research might be conceivable.

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Additional Literature

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Links on the Web

- Lindel Research Group Marine Natural Products – Tools of Life
www.oc.tu-braunschweig.de/lindel
- Hentschel Research Group Marine Sponges / Novel anti-infectives University of Würzburg
<http://www.uni-wuerzburg.de/?id=85392>