ABSTRACT
Bioactive marine natural products can be defined as biologically active products, both secondary metabolites as well as enzymes, lipids and heteropolysaccharides derived from marine sources. Marine natural products have been found to be an important source of drugs and drug leads. These natural products are secondary metabolites and enhance survival fitness and may serve as chemical weapons used against bacteria, fungi, viruses and small or large animals. Most of the natural products of interest to the pharmaceutical industry are secondary metabolites and several such compounds, derived from marine invertebrates, have been in clinical trials as experimental anti-cancer drugs. Recently, cytotoxic natural products have been extracted from marine invertebrates in the cold waters around Iceland. Extracts from the starfish Ctenodiscus crispatus and from the sponge Isodyctia showed considerable cytotoxic activity. Extensive studies have been carried out on proteolytic digestive enzymes isolated from Atlantic cod. These proteinases have higher catalytic activity, even at very low temperatures, than mammalian enzymes. These cold-adapted marine enzymes are also more temperature and acid sensitive than enzymes from conventional sources. The cold-active proteinases have many potential uses in industry, medicine and research. A large collection of psychrophilic and psychrotolerant bacterial strains have been studied. Many of these bacteria produce exoenzymes such as proteinases, lipases, amylases and other enzymes. Understanding the properties of such extracellular bacterial enzymes is important with regard to fish and fish products, since these enzymes are involved in fish spoilage.

Keywords: Cytotoxic marine products, secondary metabolites, natural products, cold-active enzymes.

INTRODUCTION
There is growing interest in marine natural products or marine secondary metabolites. This field of research receives the attention of investigators in various fields, marine biology, marine ecology, biochemistry, chemistry and pharmacology. In the industrialized countries, about 25% of all prescription drugs contain active principles that are still extracted from higher plants. Natural products have been, and still are, an inexhaustible source of drug leads as well as drugs (Korolkovas 1984). Natural products are, along with combinatorial chemistry, at the forefront of research in the search for new therapeutic agents. The explosion in novel organic compounds isolated from marine organisms is reviewed on a continuing basis by Faulkner (1988). This article will only highlight some basic principles and a few selected examples.

All forms of life use chemical reactions for maintenance, growth and reproduction. Components of the external environment, *i.e.* food, are
processed to produce cellular components and to allow for energy conservation. The many chemical reactions involved in these activities are summarized as metabolism and the participating compounds are metabolites. The smallest metabolite is the proton, but the term excludes the very large macromolecules and refers to compounds having molecular masses with an upper limit of about 1500 daltons.

Metabolites are classified into two broad types, primary and secondary. Primary metabolites are essential to growth and life in all living systems, and are formed by a limited number of metabolic reactions. Primary metabolites serve as building blocks for synthesis of macromolecules, proteins, nucleic acids, carbohydrates and lipids. Secondary metabolites are not essential to the life of the producing organism and are formed from primary metabolites. Many of the secondary metabolites enhance the survival fitness of the organism and may serve for example as chemical weapons used against bacteria, fungi, insects and large animals. Most of the „natural products“ of interest to the pharmaceutical industry are secondary metabolites, but there is also growing interest in products of primary metabolites such as various marine lipids, enzymes and complex heteropolysaccharides.

MARINE NATURAL PRODUCTS

The oceans have always provided food in abundance, but until recently marine secondary metabolites have made relatively little impact, with the exception of the marine toxins and mollusk dyes. Several materials from marine invertebrates have been in clinical or pre-clinical anticancer trials at the National Cancer Institute in the USA. They include (Fig 1) bryostatin, didemnin B, dolastatin, ecteinascidin, halichon-

Figure1 Structures of some of the bioactive marine products of interest.
drin B, and halomon (Flam 1994). Several other compounds of varying skeletal structures have also been bioassayed for antitumor activity. Juncusol from an estuarine marsh plant, aplysisatin from a sea hare and aeroplysinin-1 from sponges are only a few of the marine isolates which are undergoing further evaluation for anticancer activity. A few other marine products have already provided medicinal or agricultural products, or have served as lead compounds for chemical synthesis.

Chemical structures of marine products often differ from terrestrial secondary metabolites in being halogenated with bromine and/or chlorine. One monoterpene from a red alga has a molecular formula \( \text{C}_{10}\text{H}_{12}\text{Br}_{3}\text{Cl}_{3} \), with 50% by weight of bromine and 22.3% of chlorine (Faulkner 1995). Some of the halogenated materials have antibiotic properties; laurinterol, \( \text{C}_{15}\text{H}_{19}\text{OBr} \), from a red alga has activity against gram-positive bacteria comparable to that of streptomycin (Rinehart 1992).

Some sponges contain unusual arabinose nucleosides, for example spongo-uridine (1-D-arabinofuranosyl-uracil) and spongo-thymidine (1-D-arabinofuranosyl-thymidine), which are considered secondary metabolites (Rinehart 1992). An adenine analog, vidarabine (Ara A), of these materials was first synthesized as an anticancer agent and is also produced by a strain of \textit{Streptomyces antibioticus} and finds some use as an antiviral agent. This adenine analog has an inhibitory action on DNA polymerase, ribonucleotide reductase and adenyl cyclase.

Exploitation of marine products can present difficulties: sites may be inaccessible and tidal conditions may hinder harvesting. In at least one case, that of bryostatin, aquaculture is being used to overcome supply difficulties. This material, a complex polycyclic polyether structure, is produced by the Pacific coast bryozoan, \textit{Bugula neritina}, and the company CalBioMarine Technologies is growing colonies in 5000 liter tanks (Rouh 1995).

Some marine metabolites are actually produced by microbial symbionts of particular species. One example is tetrodotoxin (fugu poison), the neurotoxin which causes pufferfish poisoning. It has been shown that tetrodotoxin is produced by many strains of marine bacteria (Simidu \textit{et al.} 1987). It is now generally believed that tetrodotoxin is synthesized solely by bacteria and accumulates in various fish through the food web. Certain metabolites isolated from filter-feeding sponges are also probably produced by microorganisms. The paralytic shellfish poison saxitoxin has its origin from dinoflagellates. In this case the original compound (e.g., saxitoxin) in dinoflagella survives molecular modification when that organism is bioconcentrated by the host species (e.g., clam).

Various research groups in Australia, USA and in several other countries work towards pharmaceutical objectives and seek compounds with anticancer and antibacterial properties and also receptor agonists, antagonists, cardiotonic peptides and other bioactive marine natural products. Some of these compounds have been synthesized and others serve as leads in the synthesis of bioactive analogs. Recently researchers at Scripps Research Institute, La Jolla, California, have achieved the first total synthesis of eleutherobin (Fig. 1), a promising anticancer agent obtained from a Pacific Ocean coral (Nicolaou \textit{et al.} 1997). Eleutherobin has the same mechanism of action as taxol, it prevents the disassembly of cell microtubules, making it impossible for the cell to divide.

RESEARCH ON BIOACTIVE MARINE PRODUCTS IN ICELAND

Cytotoxic natural products from marine invertebrates

Recently an effort was made to search for cytotoxic natural products in marine invertebrates. The results are promising but the study was limited to a few species. Extracts from the starfish \textit{Ctenodiscus crispatus} showed considerable cytotoxic activity, as well as extracts from the sponge \textit{Isodyctia}. The identification of the bioactive compounds has not been completed. The cytotoxicity was measured in a test system employing the larvae of \textit{Artemia salina}. This is a preliminary study but an effort will be made to explore the chemical arsenal of marine life in the cold waters around Iceland.

Psychrophilic or cold-adapted marine proteinases from fish

Extensive studies have been carried out on proteolytic digestive enzymes isolated from the
pyloric caeca of Atlantic cod (*Gadus morhua*). Bjarnason *et al.* (1997) at the Science Institute, University of Iceland, have studied trypsin, chymotrypsin, elastase and collagenase. The cDNA for several of the cod serine proteinases have been isolated, sequenced and characterized with respect to their deduced amino acid sequences. Sequence alignments show high homology to their mammalian counterparts. These proteinases have higher catalytic activity, even at very low temperatures, than comparable mammalian enzymes, permitting the use of lower amounts of enzyme adjuncts in various processes. They are more temperature and acid sensitive than enzymes from conventional sources, allowing the use of milder conditions to destroy residual enzyme activities if needed, after processing is complete.

The cold-active proteinases, purified or in a mixture called Cryotin, have many potential uses in industry, medicine and research, especially in food processing applications which require hydrolysis at low temperatures, inactivation under mild conditions or native collagen digestion. The aim is to make these cold-active enzymes and potentially powerful industrial tools commonly available in the future (Bjarnason *et al.* (1997)).

**Enzymes from psychrophilic bacteria**

In the marine environment both psychrophilic and psychrotolerant gram-negative bacteria are commonly found, especially in colder waters. Many of these are producers of exoenzymes such as proteinases, lipases, amylases, and other enzymes. The extracellular proteinases excreted by marine bacteria most likely facilitate the utilization of proteins as a nitrogen source by these bacteria.

Understanding the characteristics of such extracellular proteinases is important for elucidation of the adaptation of bacteria to various marine environments. Such understanding is also important with regard to fish and fish products as these enzymes are important in fish spoilage. Proteolytic extracellular enzymes are also important as contributors to the virulence of some fish disease bacteria. These enzymes may cause direct tissue damage and may also enhance the invasiveness of some disease-producing bacteria.

During the last few years a large collection of psychrophilic and psychrotolerant proteinase-producing bacterial strains has been established by Alfredsson *et al.* (1995) at the Microbiology Laboratory, University of Iceland. Marine strains from this collection were selected for growth studies and proteinase production. Very limited information is available on the characteristics of psychrophilic bacteria, whereas extensive information is available on such enzymes from thermophiles.

Low-temperature strains were selected for further studies and one of the protease producing strains was selected for protease production, purification, characterization, and comparative studies. An enzyme purified from this strain was characterized as an alkaline serine protease, belonging to the subtilisin family. The enzyme is presently the subject of a study aimed at gaining insight into the molecular mechanisms underlying cold adaptation of enzymes (Alfredsson *et al.* (1995)).

**REFERENCES**


The effect of nutritional status of Icelandic cod (Gadus morhua) on macroconstituents and trace elements in the liver

Guðjón Atli Auðunsson
Icelandic Fisheries Laboratories
Skúlagata 4, P.O.Box 1405
IS-121 Reykjavík, Iceland.

ABSTRACT
Cod liver is widely used for monitoring inorganic and organic contaminants in the marine environment. However, the variation in its size and composition may be substantial and has hampered the full use of the vast amount of data collected in national and international monitoring programmes. Hence, there is an urgent need for a model that adequately describes the interrelationship between the levels of trace elements in cod liver and the biological and biochemical covariables that may affect these levels. It is the aim of this study to look for such relationships by examining data from Icelandic monitoring and research programmes. Icelandic waters may be considered relatively unpolluted and a priori well suited for studying the effects of natural biological and biochemical covariables on the levels of trace elements in cod liver. The present work makes use of data on cod liver from a programme on monitoring contaminants in Icelandic waters in the years 1990-1998 and additional data collected in 1994-1996, for information on possible effects of season and size of fish. In addition to biological variables, data on biochemical constitution of the livers were compiled, i.e. moisture, fat, nitrogen, phosphorus, ash, and minerals, together with data on several trace elements (Zn, Cu, Fe, Mn, Cd, Pb, As, Se). Large variability is observed of the chemical parameters analysed where the ratio of maximum to minimum values of grouped livers ranges from about three for nitrogen up to 140 for cadmium. These data reveal simple relationships between liver macroconstituents and the size of cod and its liver, enabling the macrocomposition of the liver to be defined with considerable reliability in terms of the hepatosomatic index. The model also fits data from the literature. Furthermore, variation of the lean fraction of the liver explains 90% or more of the variation of liver macro- and microconstituents, where simple linear equations adequately describe their interrelations. Finally, all the trace elements correlate fairly well with the liver lean fraction and the age of cod in a similar fashion as the macroconstituents, albeit with log-transformed data. These models may form a basis for the evaluation of micro- and trace contaminant concentrations in cod liver. The relations found are of help in the interpretation of data from Icelandic waters, but the models need not apply for other waters. The effect of biological and biochemical covariables on trace element concentrations in cod livers from other areas is a prerequisite if meaningful spatial comparisons are to be performed. The same is true when temporal trends in a given area are studied. It is suggested that a common basis for spatial and temporal comparisons of cod livers be a hypothetical cod liver of 100% lean fraction from one year old cod.

Keywords: Cod, cod liver, monitoring, trace elements, macroconstituents, biological covariables, biochemical covariables, modelling.

Dedicated to Professor Unnsteinn Stefánsson in honour of his contributions to oceonography and education.