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Studies on marine toxins: chemical and biological aspects

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Abstract. The structures and mechanisms of biological action of the best known representatives of the main groups of marine toxins are presented. It is shown that many compounds have complex chemical structures and possess extremely high toxicities. Characteristic features of isolation, structure determination and syntheses of these compounds using the achievement of modern organic chemistry are discussed. The methods of identification and quantitative analysis of marine toxins are briefly reviewed. The bibliography includes 147 references.

I. Introduction

Studies on low-molecular-weight marine natural compounds acting as bioregulators is a quickly developing field of bioorganic chemistry at the interface between chemistry and biology. Initially, such investigations have been the prerogative of organic chemistry. Then, as the elucidation of the biological role of these compounds has begun together with the development of trends towards molecular biology associated with the examination of the biosynthesis and mechanisms of physiological action of marine metabolites, they became a part of biological sciences to an increasingly greater extent. To date, this field of research has not lost connections with chemistry and currently exerts a considerable influence on the development of organic and analytical chemistry.

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Advances in the study of marine toxins (*i.e.*, toxins isolated from marine organisms) are a good illustration of this statement. The present work attempted to examine these achievements, placing an emphasis on those having stimulated both studies of synthetic chemists and investigations at the interface of chemistry and biology employing state-of-the-art methods of substance identification. Examples are mainly taken from the practice of the last 10 – 15 years, although mainframe studies of the earlier period are also cited. The references provided are, for the most part, review articles concerning selected groups of marine toxins or selected directions of their study.

The objective of our study, which does not pretend to be a comprehensive review on any of the aspects mentioned, is to draw the attention of national chemists to marine toxins and analyze the major achievements in this field of science.

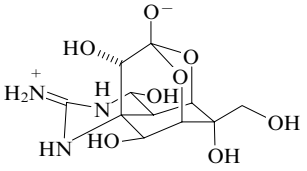
II. A brief overview of marine toxins

Terrestrial organisms including some higher plants and pathogenic bacteria had been for a long time regarded as the exclusive primary biological sources of toxins. After the invention of scuba diving equipment, increase in seafood production and progress in its cultivation, many toxic marine organisms also gained some popularity. The study of marine organisms appreciably broadened the scope of known natural compounds, particularly toxins. At present, the total number of natural compounds with determined structures does not exceed 150 000 – 200 000, and around 20 000 of them were found in marine organisms. Among a hundred of toxic secondary metabolites, there is quite a few extremely active compounds. Here we present some data on low-molecular-weight (molecular weights < 3500 Da) toxins of marine microorganisms and invertebrates, many groups of such compounds being represented only by examples of the most toxic compounds. The protein toxins of Coelenterata, some fishes, sea snakes and other marine organisms remained beyond the scope of this review.

1. Toxins produced by marine microorganisms

There are several aspects that attract special attention to a variety of toxins of microbial origin. Firstly, toxins produced by marine microorganisms are transferred through food chains to various macroorganisms including edible ones. Accumulation of toxins in the latter can turn them dangerous for humans. This determines the importance of the development of highly sensitive methods for the identification and quantitative analysis of marine toxins and the necessity for continous monitoring of the appearance and blooming of toxic microorganism species in different waters of the ocean. Secondly, it is the compounds of this group that are most potent among all the known non-protein toxins. Thirdly, from the chemical point of view, many of them have unusual and complex structures [1] and challenge modern science in both their structure determination, including stereochemistry, and the development of approaches to their syntheses.

Of marine toxins studied by chemists, the first one was tetrodotoxin (TTX, **1**) isolated in 1909. It is accumulated in the gonads and liver of the so-called pufferfish (the Tetraodontidae family).



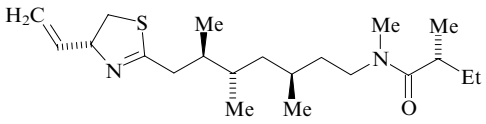
1

Later this compound has been identified in the freshwater newts of the genus *Taricha*, toads of the genus *Atelopus*, marine nemertean worms, several species of crabs, octopi and other biological objects.[2,3] It is known that some of poisonous fishes, particlularly, fugu (*Takifugu* or *Spheroides rubripes*) is delicacy in Japan, despite not infrequent cases of fatal poisoning. The dose of tetrodotoxin that kills 50% of animals tested (LD_{50}), is $\sim 8\ \mu g\ kg^{-1}$ when administered intravenously and $\sim 330\ \mu g\ kg^{-1}$ orally, *i.e.*, tetrodotoxin is nearly 100 times more toxic than potassium cyanide. The principal cause of human deaths is respiratory paralysis.

Tetrodotoxin (**1**) is a specific blocker of the gating mechanism of some sodium channels (the so-called TTX-sensitive channels), which impairs nerve conduction.[4] Its structure was eludicated in the classical studies by Woodward (USA), Tsuda (Japan) and Goto–Hirata's group (Japan).

It was established that tetrodotoxin is synthesized by some bacterial species (*Pseudoalteromonas tetraodonis, Vibrio alginolyticus*). It is by this fact that its isolation from a number of phylogenetically unrelated biological objects is accounted for.[5] It is assumed that toxin-producing bacteria protect sexual products of fish from being eaten by predators after they have been shed in seawater during spawning. In a number of works, the identification of this toxin in various marine bacteria was carried out mainly by chromatography of the alkaline degradation products of tetrodotoxin followed by silylation. This approach is not sufficiently reliable and positive results can be obtained even for the nutrient media used for culturing of the bacteria.[6]

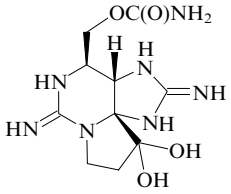
Marine cyanobacteria produce also other, less active toxins. Many of them are neurotoxins, tumour promoters and liver toxins. Recent research in this area can be exemplified with kalkitoxin (**2**), a compound isolated from a Caribbean collection of colonies of the cyanobacterium *Lyngbya majuscula*.[7]



2

The absolute configurations of its five asymmetric centres and its complete structure were determined after the synthesis of four stereoisomers of kalkitoxin, one of which proved to be identical to the natural product. The ichthyotoxicity of this compound **2** is very high (the concentration of approximately 600 pg per ml of seawater is lethal). This compound was not inferior to tetrodotoxin in its ability to block the sodium channels of mouse neurons.

Yet another marine toxin, saxitoxin (**3**), is also well-known.



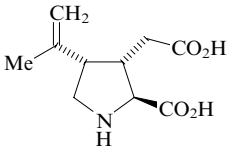
3

This compound and nearly 20 its natural analogues and derivatives discovered to date (for instance, neosaxitoxin, decarbamoylsaxitoxin, gonyautoxins) induce paralytic shellfish poisoning (PSP).[8] The main toxin of this series was named after the clam *Saxidomus giganteus* from which it was isolated for the first time.[9] In the USSR in the early 1980s, several fatal poisonings of this type were registered in the Kamchatka region upon eating mussels collected at the Avacha bay coast. The main toxin isolated at that time was identified as 11-hydroxysaxitoxin sulfate.[10]

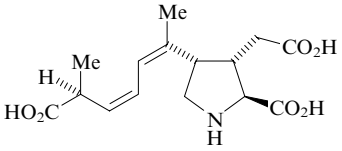
PSP-Toxins get into planktivorous molluscs along with dinoflagellate microalgae during mass development of the latter and accumulate in some organs and tissues of these molluscs. They were also found in some fishes, in particular, in pufferfish.[6] Many countries monitor the development of potentially toxic microalgae as well as the content of toxins in shellfish tissues and impose a ban on shellfish harvesting during dangerous periods. A great deal of microalga species that can have PSP-toxic strains are known. Among them are representatives of the genera *Alexandrium*, *Gymnodinium* and *Pyrodinium*. In addition, toxins of this group were discovered in the *Aphanizomenon flos-aquae*, *Anabaena curcinalis* cyanobacteria, *etc*. It is supposed that paralytic shellfish toxins, like tetrodotoxin, are of bacterial origin. For example, saxitoxin was found in the bacterium *Moraxella* sp. culture isolated from *Alexandrium tamarense* (or *Protogonyaulax tamarensis*).[11]

In its mechanism of action and toxicity, saxitoxin (**3**) resembles tetrodotoxin and, like the latter, inhibits sodium conduction across nerve cell membranes. The poisoning symptoms in humans include numb lips, gastrointestinal

disorders, and, in very bad cases, respiratory failure. Two toxins containing a pyrrolidine ring, *viz*., kainic (**4**) and domoic (**5**) acids, were first detected in macroalgae.



4



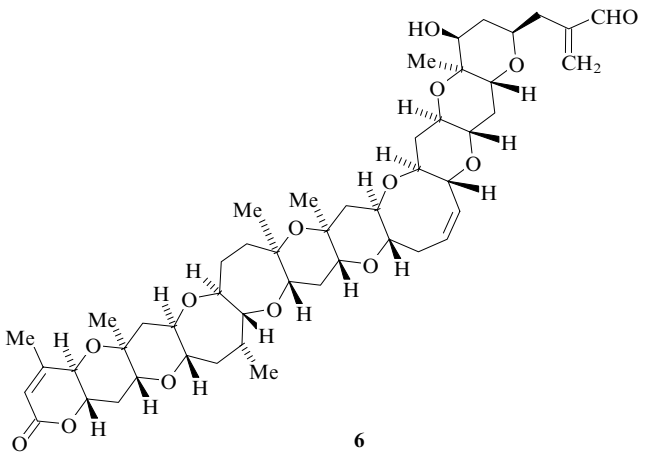
5

Kainic acid (**4**) was isolated from the red alga *Digenia simplex*, which has been used since old times in Japan as an anthelmintic, and the related acid **5** was obtained from the red alga *Chondria armata*.[12] The name of compound **5** is derived from the Japanese name of *Chondria armata* 'domoi'.[13] Later it was found that compound **5** is the cause of the so-called Amnesic Shellfish Poisoning [ASP-toxicity or DAP- toxicity (Domoic Acid Poisoning)]. It was revealed that the diatoms of the genus *Pseudo-nitzschia* as well as *Nitzschia navis-varingica* and *Amphora coffeaeformis* can be producers of this toxin.[13] However, the existence of both toxic and non-toxic strains is typical of the majority of the *Pseudo-nitzschia* diatoms. The difference in the ability of these strains to produce acid **5** can result from both genetic variance and the influence of environmental factors. All the strains must contain genes responsible for the production of this toxin. However, these genes have not been identified yet.[14]

Poisoning with shellfish impairs functions of the central nervous system and violates memory.[15,16] The first instance of such a poisoning with the mussel *Mytilus edulis* was reported in 1987. The toxic effect is associated with an interaction of compound **5** with the glutamate receptors of brain cells.[17] Domoic acid results in almost permanent stimulation of hypothalamus neurons and ultimately brings on their destruction.[13] It was established that acid **5** in subtoxic doses and salts of aspartic and glutamic acids, which are normally present in mussel tissues, enhance effects of each other leading to the increase in toxicity. The victims exhibit symptoms that are characteristic of Alzheimer's disease, and the most severe poisoning, up to the fatal cases, has been observed for the elderly people.

Domoic acid (**5**) is dangerous not only for humans, but also for birds and marine mammals feeding on seafood. For example, at the coast of California, pelican, cormorant and sea lion death instances associated with blooms of toxic diatoms were reported.[13]

Neurotoxic shellfish poisoning (NSP-toxicity) also results from the accumulation of toxins of microalgal origin.[18] One links the action of these toxins to the deaths of a variety of animals (from bottlenose dolphins to birds) as a result of blooms of the dinoflagellate *Karenia brevis* on the coast of Florida (USA) and raphidophyte microalga *Chattonella* cf. *verruculosa* in the waters of the north Atlantic coast of the US. Toxins of the NSP-type were called brevetoxins, about a dozen of them are known in total, including brevenol A, brevenol B, brevenal, *etc*. Brevetoxins [for example, brevetoxin-B (**6**)] belong to the type of polyether compounds. Like other metabolites of this class, they bear a system of fused oxygen-containing rings.[19]



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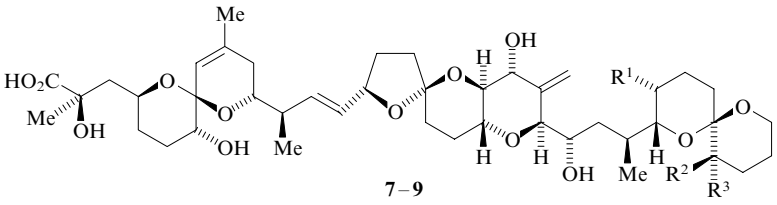
Brevetoxins activate sodium channels and give rise to a membrane depolarization of excitable neurons and unstriped muscle cells. When poisoned with such toxins, people, as a rule, do not die, however, they exhibit a whole series of gastrointestinal and neurologic symptoms including nausea, vomiting, muscle pain, loss of coordination, paresis of the facial muscles and partial paralyses. Aerosols formed during storms and hurricanes when *K. brevis* is blooming irritate the respiratory tract and make Florida inhabitants suffering from allergic manifestations and asthma attacks.[20] One of the implications of the action of brevetoxins can be weakening of the immune system of animals, which subsequently die from infections and other secondary diseases.

The so-called Diarrhetic Shellfish Poisoning (DSP-toxicity) is brought on by a whole series of microalgal toxins, among them are okadaic acid (**7**), dynophysistoxins (**8**, **9**), pectenotoxins (for instance, compounds **10**, **11**) and yessotoxin (**12**). They also have polyether structures and, like other above-mentioned toxins, can accumulate in shellfish.

Okadaic acid (**7**) was first isolated from the *Halichondria okadai* and *H. melanodoca* sponges.[21] Nevertheless, later it was established [22] that it gets into the sponges together with dinoflagellates of the *Prorocentrum* and *Dinophysis* genera. The microalgae *Prorocentrum* spp. often enter symbiotic relationships with a whole set of benthic organisms including macrophytic algae. Through food chains, compound **7** penetrates into not only planktivorous molluscs, but also other marine organisms up to fish.

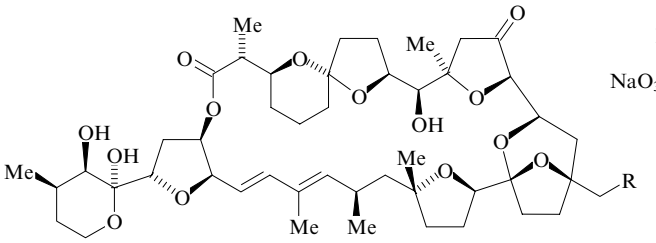
The ability to inhibit protein phosphatases due to the interaction with the so-called PP1- and PP2A-catalytic domains underlies the mechanism of the physiological action of compound **7** responsible for a part of the diarrhetic poisonings. This toxin inhibits human and animal enzymes as well as yeast and plant protein phosphatases equally successfully. The half maximal inhibitory concentrations (IC_{50}) of the PP-2A and PP-1 type protein phosphatases are approximately 0.2–1.0 and 60–500 nmol litre^{-1}, respectively.[23] In addition, okadaic acid (**7**) is a powerful tumour promoter.[24]

Dynophysistoxins **8** and **9** are similar to okadaic acid in the structures and properties, the former is its methyl homologue and the latter is its isomer. Recently, the stereochemistry of compounds **8** and **9** isolated from the dinoflagellate *Dynophysis* spp. has been elucidated.[25]



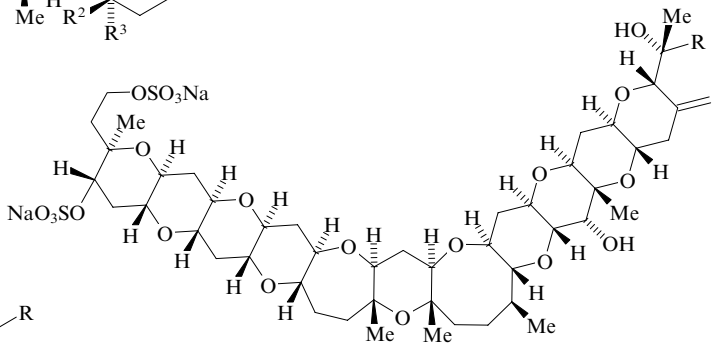
7–**9**

7: R^1 = Me, R^2 = R^3 = H; **8**: R^1 = R^2 = Me, R^3 = H;
9: R^1 = R^2 = H, R^3 = Me.

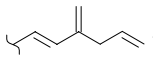


10, **11**

10: R = OH; **11**: R = H.



12

R = .

In European molluscs, okadaic acid occurs more often, while in those collected at the coasts of Japan and Canada it is dynophysistoxins. Stereochemical features of compound **8** determine its relatively weak inhibitory effect on the PP2A protein phosphatase.

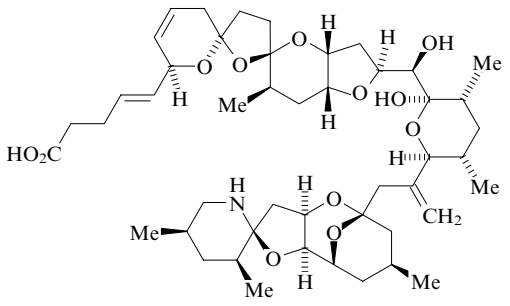
Pectenotoxins, in particular, pectenotoxins-1 (PTX-1, **10**) and -2 (PTX-2, **11**)[26] are traditionally attributed to diarrhetic toxins since they usually accompany okadaic acid. They were isolated from the microalga *D. acuminata*. To date, nearly 15 compounds of this type are known. All of them as well as quite a number of powerful cytotoxins (amphidinolides, *etc.*), are macrocyclic lactones (macrolides). Pectenotoxins are often found (following blooming of *Dinophysis* and related dinoflagellates) in edible and cultured molluscs, for example, in the Mediterranean mussel *Mytilus galloprovincialis*. When admistered to mice, these compounds do not bring about diarrhoea, but they are highly hepatotoxic. At the cellular level, pectenotoxins trigger disruption of the actin filament cytoskeleton. Moreover, they are stronger apoptotic agents towards tumour cells than towards normal cells of the same tissue.[27]

Yessotoxin (**12**) and its first analogues were isolated from the digestive glands of the toxic scallops *Patinopecten yessoensis* collected in 1986 and 1993 in Japan.[28] The toxicity of these substances is within the limits of 20 to 500 μg kg^{-1} (for mice on intraperitoneal administration).[28,29] To date, almost 40 compounds of this class are known.

Yessotoxins are produced by the dinoflagellates *Protoceratium reticulatum*, *Lingulodinium polyedrum* and *Gonyaulax spinifera*. Like dynophysistoxins, they do not bring about diarrhoea and, in addition, do not inhibit protein phosphatases. The precise mechanism of their physiological action is currently unknown. An opinion was expressed[30] that the attribution of these compounds to diarrhetic toxins is erroneous.

Azaspiracid shellfish poisoning has been known since 1995. Initially, toxins of this group (spirolides) have been found in the mussels *M. edulis* cultivated in Ireland,[31] then in shellfish from Morocco and a number of countries of western and nothern Europe (France, United Kingdom, Norway, *etc.*). It is supposed that these substances are produced by the dinoflagellate *Protoperidinium crassipes*.[32] Recently, a new microalgal species, *Azadinium spinosus gen. et sp nov.*, was identified as the primary producer of azaspiracids.[33]

Azaspiracids structurally also belong to polycyclic polyether derivatives, one of spirocycles containing a nitrogen atom. The stereochemistry of these compounds was established as late as 2006 when Nicolaou *et al.*[34] have accomplished total synthesis of azaspiracid-1 (**13**) and obtained several of its degradation products.

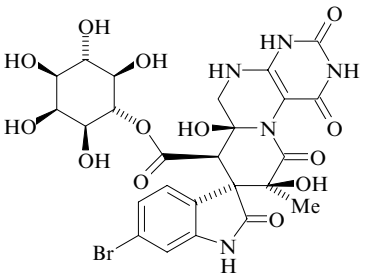


13

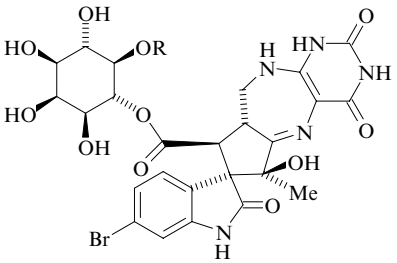
Azaspiracids are highy toxic to humans and animals, their LD_{50} are ~110–200 μg kg^{-1} (for mice on intraperitoneal administration);[35] in humans, they bring about injuries of gastrointestinal and lymphoid systems.[36]

In September 1965, mass poisoning of people living on the Suruga bay coast in Japan was observed. The poisoning was caused by the consumption of the ivory shell *Babilonia japonica*. The isolated toxins were called surugatoxin (**14**) and neosurugatoxin (**15**).[37]

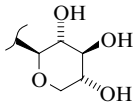
In 1985, the isolation of yet another substance of this series was reported. It received the name of prosurugatoxin (**16**) since it was readily produced from neosurugatoxin (**15**) as a result of the elimination of the terminal xylopyranose upon treatment with dilute acetic acid.[38] Particularly hazardous are neosurugatoxin and prosurugatoxin, which cause visual and speech impairments, paralysis of the facial muscles and a number of other symptoms in humans. Like atropine, both these compounds are mydriatics, giving rise to dilated pupils as a result of binding to the nicotinic receptors of neurons.



14



15, **16**

R =  (**15**), H (**16**).

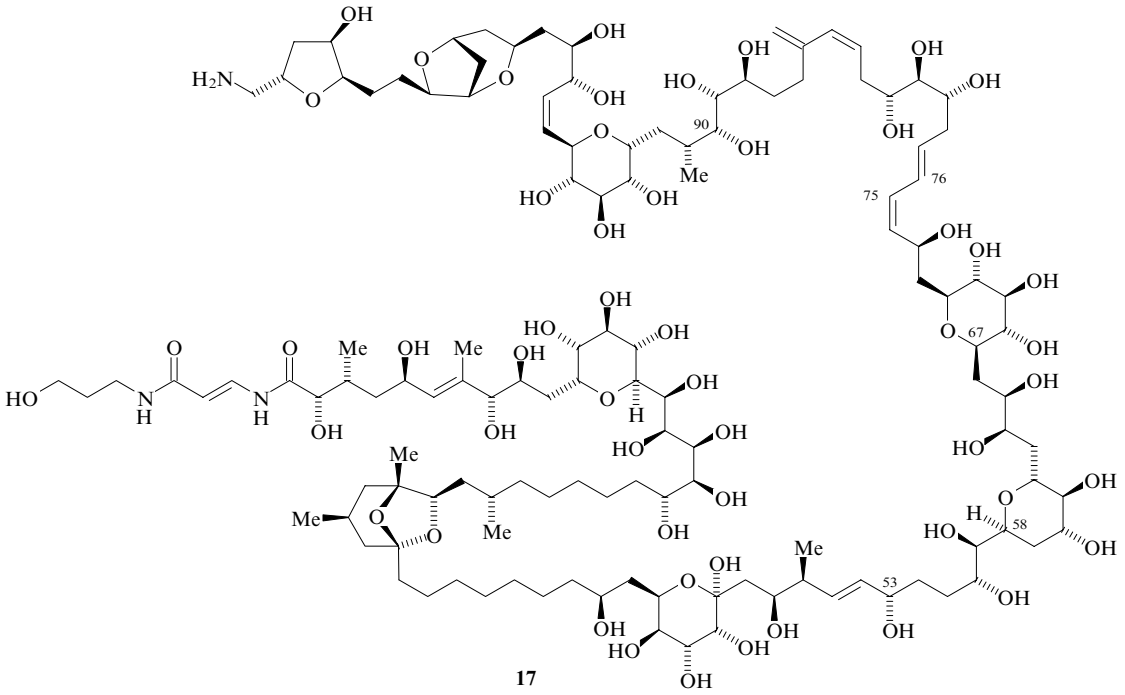
Neosurugatoxin is nearly 10 times as active as atropine. Its ability to interact with nicotinic receptors is more than 1000-fold higher than that of the pharmaceutical mecamylamine. This type of shellfish activity is also related to the accumulation of toxins from microorganisms, *viz*., from coryneform bacteria.

Some other structurally complex marine metabolites (of the polyether type or those that can hardly be attributed to a particular structural type) are also highly toxic. Thus palytoxin (**17**) was first isolated by Moore and Scheuer [39] from the Hawaiian Zoanthid † *Palythoa toxica*. Its toxicity is nearly 10 000 times that of potassium cyanide, and LD_{50} is ~ 0.15 μ kg^{-1}. This toxin comprises 129 carbon atoms, has no repeating fragments and contains more than 60 chiral centres. The elucidation of the palytoxin structure carried out by two American and one Japanese research groups in the early 1980s [40–42] became one of the major advances in bioorganic chemistry, and the subsequent synthesis of palytoxin carboxylic acid has been one of the most impressive studies in the area of organic synthesis over recent years.[43, 44]

† Zoanthids or Hexacorallia inhabit many tropical zones of the ocean.

Investigation into the mechanism of action of palytoxin as well as other aspects of its study led to a whole series of outstanding scientific achievements. It was established that it interacts with the Na^+,K^+-ATPase of different cells including cardiocytes. This interaction occurs at approximately the same site as in the case of known plant toxins, the so-called cardiac glycosides, for instance, with ouabain. Having treated with palytoxin, the most important pump cell system (ATPase), which maintains the gradient of ion concentrations on both sides of the biological membranes, turns into an ion channel prodiving an increase in sodium conduction and a possibility of the influx of the calcium ions to the cells.[45, 46] A recent study of the conformational features of palytoxin demonstrated that in aqueous solutions it forms a dimeric supramolecule, which interacts with Na^+,K^+-ATPase.[47]

In nature, palytoxin is presumably synthesized by the dinoflagellates of the *Ostreopsis* genus that are symbionts of Zoantharia. In order to prove whether this assumption holds for its analogues ostreocins, the latter were isolated from cultured microalgae (the dinoflagellate *Ostreopsis siamensis*).[48] According to evidences from other studies,[49] one cannot assert with certainty that this species is the primary producer of palytoxin since the toxicity level in Zoantharia is not related to the presence of the symbiont algae. This toxin was also found in other marine animals, for example, crabs and sea anemones.[50] Numerous instances of human poisoning caused by compound **17** and related toxins are documented.

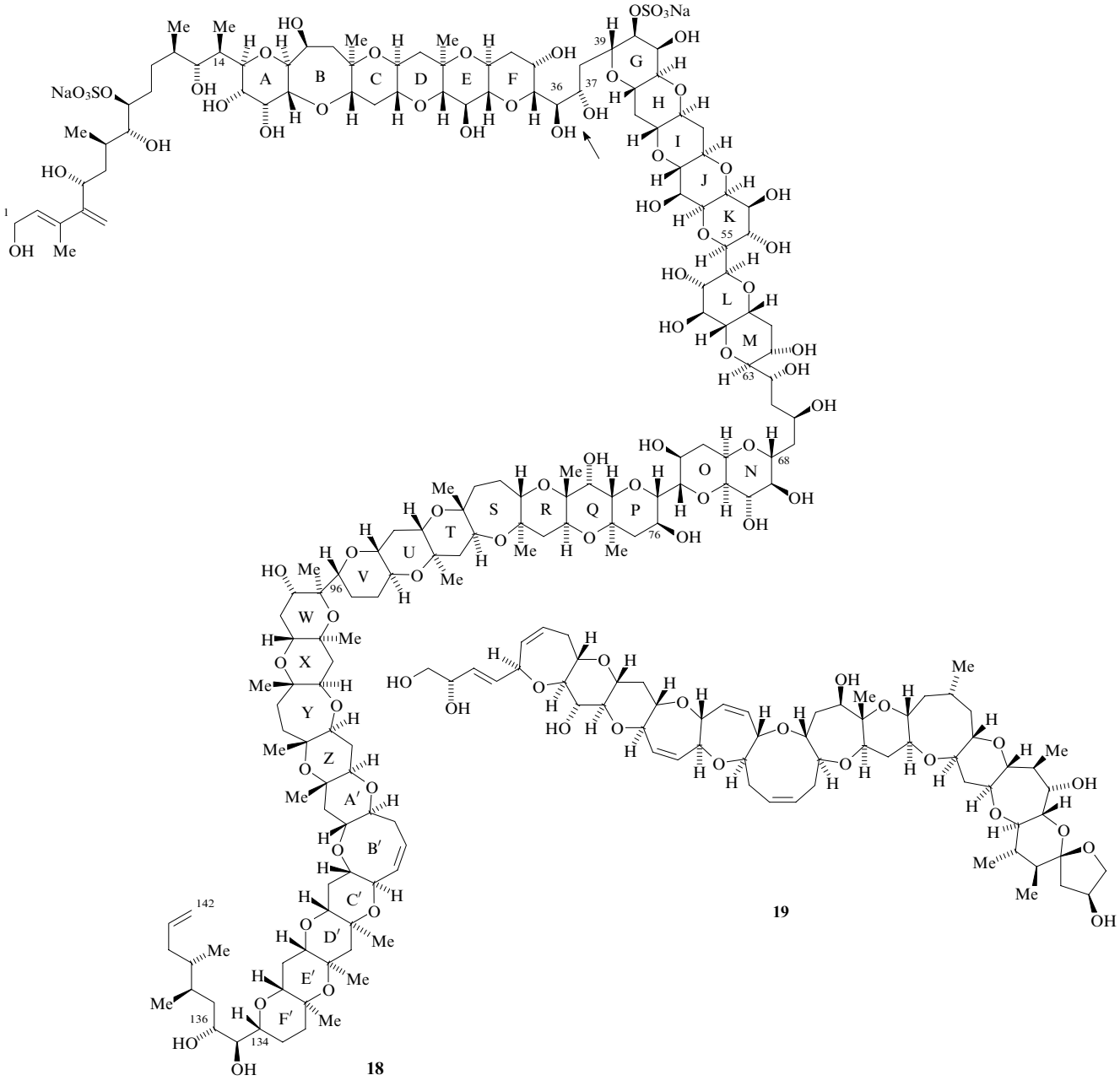


17

The so-called ciguatera-toxicity of tropical fishes is yet another type of toxicity manifested by marine organisms. This phenomenon consists of the fact that edible fishes sometimes turn toxic and even highly toxic and their consumption results in severe, up to fatal, poisonings. The ciguatera-toxicity has been known for about 200 years, however only in the second half of the 20th century it began to manifest itself in virtually all parts of the world, which was associated with a rapid increase in fishery in tropical seas. Up to 10 000 – 20 000 people annually suffer from such poisonings.[51] The toxins get into fish tissues through food chains: from poisonous dinoflagellate microalgae, some surface-dwelling algae, to algivorous fishes and then to predatory fishes. The ciguatera-toxicity is also characteristic of a number of edible fishes, for example, barracuda, soapfish and eel. Depending on water solubility, two types of toxins are distinguished: insoluble (ciguatoxins) and soluble (maitotoxin).

Maitotoxin (**18**) is the most toxic and structurally complex one of all the known non-protein toxins. Its molecule contains 142 carbon atoms and 68 oxygen atoms (excluding those of the sulfate groups) as well as 32 oxygen-containing rings. Its structure was elucidated by Japanese and American chemists by using state-of-the-art NMR spectroscopy, mass spectrometry and synthesis of model compounds.[52, 53] The toxicity of this compound exceeds that of palytoxin severalfold (in mice, $LD_{50} \approx 50$ ng kg^{-1}). The name of this toxin was derived from the word 'maito' used by Tahiti inhabitants for the surgeonfish *Ctenochaetus striatus* from which it was isolated. It is believed that the producer of compound **18** is the dinoflagellate *Gambierdiscus toxicus*, which is found as an epiphyte on coral reef algae. Toxin **18** was obtained in the amount sufficient for its structural study by culturing this microalgae.

The high toxicity of compound **18** is explained by its ability to increase calcium influx and the efflux of the sodium ions from the cells. Maitotoxin affects calcium-



18

19

permeable non-selective ion channels (CaNSC) of excitable and non-excitable cells. This brings about the activation of calcium-sensitive proteases, calpain-1 and -2, formation of large pores in cells and so-called oncotic cell death due to calcium overload.[54, 55]

Ciguatoxin (**19**) was isolated from a poisonous fish (from the eel *Gymnothorax javanicus*), and a close analogue of this compound was isolated from the cultured dinoflagellate *Gambierdiscus toxicus*.[56]

Several groups of toxins that are similar to ciguatoxin are known (ciguatoxin-3C, gambierol, gambiertoxins, gymnocin A, *etc*.). The cyclic system of ciguatoxin consists of 12 fused oxygen-containing rings and a spiro-fused tetrahydrofuran ring. In its analogues, a smaller number of rings is found. Substances that are structurallly similar to ciguatoxin are more hydrophobic than maitotoxin because they normally contain no more than five hydroxyl groups.

Ciguatoxins were found in the dinoflagellate *Gambierdiscus toxicus*, *Coolia monotis*, *Amphidinium carterae* as well as in the microalgae of the genera *Prorocentrum*, *Ostreopsis* and *Thecadinium*. They are highly toxic, on intraperitoneal administration to mice the LD_{50} values lie in the range of $0.25-2.3$ μg kg^{-1}. The toxic effect of the ciguatoxin itself (**19**) is related to its ability to activate voltage-gated sodium channels, in large doses it blocks the function of potassium channels impairing the conduction of a nerve impulse.[57] The symptoms of poisoning by this substance in humans include gastrointestinal disorders and nervous and cardiovascular system impairments. Nausea, headache, dizziness, numb extremities are observed most frequently, in severe cases respiratory paralysis and death.[58]

Thus, diverse and structurally very complex toxins are produced by both procaryotic microorganisms (marine bacteria) and eukaryotes (microalgae). Many of them belong to extremely toxic natural compounds. For example, maitotoxin is so active that merely several kilogrammes of this substance is the lethal dose for all the population of the Earth. The majority of marine microbial toxins, being highly specific ligands, interact with specific fragments of the ion-conductive channels and pump systems of biomembranes.

2. Toxins of marine invertebrates

Some marine invertebrates are also known by their toxicity. Some of them have glands or cells where they accumulate toxins used for hunt and producer protection. Others have no such organs and contain toxic metabolites in their tissues. Not only molluscs, but also some other invertebrates can accumulate powerful toxins of microbial origin in their bodies (tetrodotoxin, palytoxin, *etc*.) as was discussed in the previous Section. Such toxins were found, for instance, in zoanthids, crustaceans, marine worms and other invertebrates.

The venom glands and toxin-producing cells of marine invertebrates usually contain proteinic or peptidic toxic compounds. They are characteristic of several groups of invertebrates, in particular, many taxons of Coelenterata (the phylum Cnidaria), some taxons of molluscs (the phylum Mollusca), sea urchins (the class Echinoidea, the phylum Echinodermata), marine worms (the phylum Nemertea). The majority of the toxins detected in these invertebrates are high-molecular-weight compounds and hence are not covered in this review.

Low-molecular-weight toxins were found in molluscs belonging to the genus *Conus*. These molluscs are predatory animals that eat fish, marine worms or other molluscs. Some species specializing in hunting fish, for example, *C. geographus* represent lethal risk also for people collecting molluscs on coral reefs.

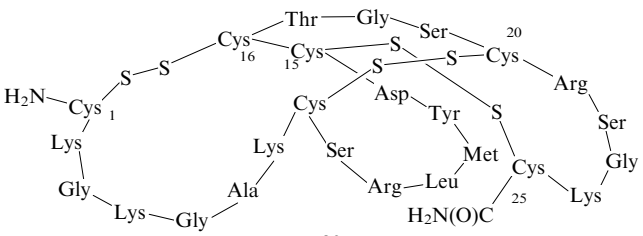
All molluscs of the genus *Conus* have poison bulbs connected by a duct to a harpoon used for predation. Currently, around 500 species of these molluscs are known. It is believed that there is a total of not less than 700 species in the ocean. In the poison bulb of each *Conus* species, there is a characteristic set of toxic peptides (the so-called conopeptides) comprising roughly one hundred compounds, and the same substance in different species can rarely be found. Thus, the diversity of conopeptides in these animals is very high.

The conopeptides rich in disulfide bonds are conventionally refererred to as conotoxins. They contain four to ten cysteine residues and two to five disulfide bonds, have low molecular weights and comprise $12-25$ amino acid residues. This distinguishes conotoxins from the polypeptidic toxins of other animals, which normaly consist of $40-80$ amino acid residues. Typical structural features of conotoxins also include high abundance of cysteine residues and the presence of a large series of post-translationally modified amino acids. Both ubiquitous modified amino acids (hydroxyprolines, glycosylated serine and threonine) and comparatively rare ones, *e.g*., 6-bromotryptophan, D-amino acids, γ-carboxyglutamic acid, sulfated tyrosine were found.[59]

Conotoxins are divided into superfamilies based on the number of cysteine residues and their distribution along the polypeptide chain. For instance, the M superfamily comprises toxins in which cysteine residues (C) are distributed according to the scheme CC−C−C−CC (the '−' character stands for other amino acid residues between the cysteine residues), while the O superfamily comprises toxins with the C−C−CC−C−C motif. The following superfamilies are known: O, M, A, S, T, P, I. In each superfamily of conotoxins, there are groups (families) that are conventionally designated by Greek letters. For example, the O superfamiliy is divided into the δ, μO, κ and ω families.[59] These families differ from one another in their ability to bind with different molecular targets. Thus the conotoxins of the δ and μO families interact with sodium channels, the representatives of the κ family interact with potassium channels, whereas the representatives of the ω family interact with calcium channels.

All the conotoxins exhibit specificity in the interaction with ion channels or membrane receptors. Different conotoxins not just 'recognize' one or another type of ion channels, but also 'select' only certain subtypes and isoforms among them. This makes conotoxins useful tools for studying the subtle mechanisms of ion conduction and related phenomena, *e.g*., conduction of nerve impulses, the onset and block of pain, *etc*. In addition, such compounds used both directly and as model compounds play an important role in the quest for novel drug candidates.[60, 61]

Pharmacological studies of various conotoxins and their synthetic analogues demonstrated that many of them are of interest as potential drugs. One of conotoxins, namely ω-conotoxin MVIIA (**20**), showed good results in all the phases of clinical trials.



20

Compound **20** was first obtained from the Pacific mollusc *Conus magnus*.[62,63] It is a linear peptide consisting of 25 amino acid residues. Six cysteine residues in this compound form three disulfide bonds. The disulfide bonds impart ω-conotoxin a spatial structure that is capable to specifically block the function of NVSCC-calcium channels (N-type voltage-sensitive channels). As a result, the toxin inhibits pain signal transmission, being very efficacious (K_d = 9 *p*M). The synthetic ω-conotoxin was called ziconotide. It turned out to be approximately 1000 times more powerful analgesic agent than morphine. Clinical trials demonstrated its high efficacy for the treatment of patients with chronic severe pains. Ziconotide, as opposed to morphine, has no hallucinogenic effect and is not addictive. It is approved for clinical use and marketed in the US as a powerful analgesic agent under the trademark name Prialt.[64]

Holothurians (Holothurioidea) is a class of echinoderms (the phylum Echinodermata) represented for the most part by soft-bodied slow-moving animals lacking mechanical protectors. However, they successfully survive, and at great depths are ones of the most numerous benthic animals. Some representatives of the order Aspidochirotida (the family Holothuriidae) from this class of echinoderms have specialized glands (the so-called Cuvierian organs). The latter are sticky tubules containing large quantities of triterpene glycosides that are highly toxic to fish. On approach of a predatory fish or mechanical irritation, these animals expel these tubules through the anus. In marine aquaria, this brings about immediate death of the fish due to hemolysis in gill capillaries, and in natural environment it repels the predator. Iinhabitants of some coral islands have used holothurians since long ago to stun fish in small lagoons by squeezing their insides containing the glycosides in water.

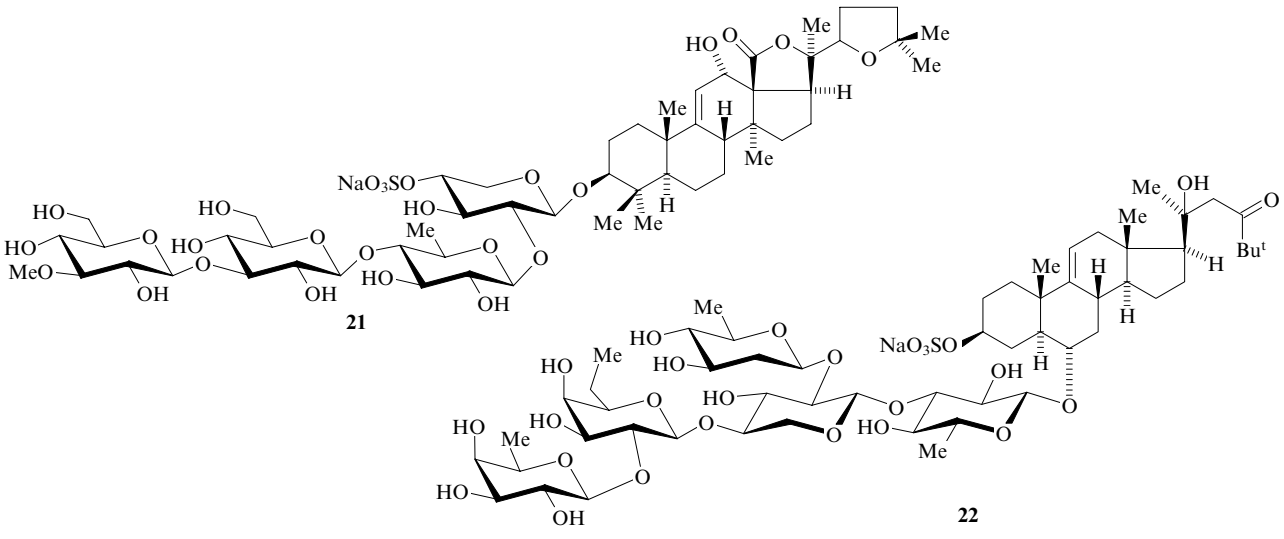
Some holothurian species have no Cuvierian organs, however, the muscle walls of their bodies contain highly hemolytic glycosides in sufficient quantities. Some holothurians (the so-called trepangs) are edible and even harvested species, and after the appropriate cooking they are considered as specialities and healthy food.

One of the first holothurian ichthyotoxins studied was holothurin A (**21**)[65] isolated from holothurians of the genera *Holothuria* and *Actinopyga*.

Holothurin from *Holothuria vagabunda* at a dilution of 1 : 100 000 kills various fishes within several minutes. Its LD_{50} for mice is 0.75, 70 and 400 mg kg^{-1} when administered intravenously, intraperitoneally and orally, respectively. Accordingly, highly toxic on intravenous administration, these substances manifest low toxicity if used for food (as part of edible holothurians). The hemolytic propetries of holothurin and other holothurian glycosides (more than 200 such compounds are known, and the majority of them were discovered by Russian scientists[65,66]) pertain to their ability to interact with cholesterol of the plasma membrane of red blood cell followed by the formation of ion-conduction pores. Analogously, these substances act on other cells containing membrane sterols, for example, on tumour cells, pathogenic fungal cells and the developing sea urchin eggs.[67]

Representatives of another class of echinoderms, starfish or sea stars (Asteroidea), use a similar strategy of chemical defence against predators. The contain (mostly in their outer tissues[68]) compounds referred to as asterosaponins. In their nature, they are also glycosides, being sterol oligoglycosides rather than triterpene glycosides.[69] Asterosaponins, for instance, thornosteroside A (**22**) from the starfish *Acanthaster planci* (known as crown-of-thorns), are weaker hemolytics than holothurian glycosides. Nevertheless, they are sufficiently highly toxic to many sea creatures.[70] These substances at a concentration of 1 – 10 μg ml^{-1} are usually toxic to fish. Their hemolytic properties are connected with both the structure of the steroid aglycone and the composition and length of the carbohydrate chain.[71]

Many starfish species are predators themselves and prey upon marine invertebrates, first of all, molluscs. Some prey animals can detect the approach of a starfish discharging asterosaponins into the surrounding water owing to speci-

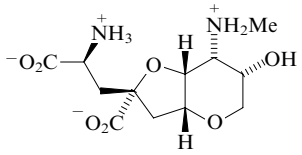


21 **22**

alized receptors transmitting the signal to the nervous system upon interaction with these toxins.[72]

A variety of low to moderately toxic low-molecular-weight natural compounds were found in a large number of sponge, ascidian and bryozoan species. In most cases, their acute toxicity was not studied on animals, instead, only their cytotoxic properties were explored. Several thousand cytotoxic secondary metabolites of marine invertebrates have been discovered. Some of them underwent pre-cilnical and then clinical trials as potentially active substances of novel antitumour drugs. They include, for instance, alkaloids from ascidians, nucleoside derivatives and macrolides from sponges, macrolides from bryozoans and many others. As a result of this research, trabectidin was discovered and in 2007 its marketing under the trade name Yondelis began. It is approved for clinical use in the EU countries for the treatment of soft tissue sarcomata. Its active principle is ecteinascidin-743 (ET-743), an alkaloid isolated from the ascidian *Ecteinascidia turbinata*, the toxic concentration of which for the tumour cells L-1210 was very low (IC_{50} = 0.5 ng ml^{-1}). The examination of a vast body of data obtained in the study of supercytotoxins of marine invertebrates is beyond the scope of this work, and the interested reader is referred to a recent review[64] and references cited therein.

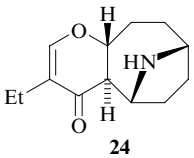
For some sponge metabolites, toxic and neurotoxic properties were studied. For example, dysiherbaine (**23**) from the Micronesian sponge *Dysidea herbacea* is a new amino acid that interacts with non-*N*-methyl-D-aspartate (non-NMDA) type glutamate receptors of the central nervous system (CNS).



23

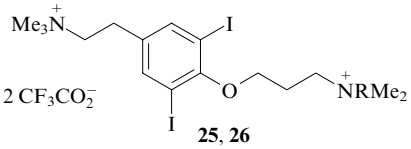
Dysiherbaine resembles domoic acid in its effect on animals since it inhibits binding of kainic acid to the above-mentioned receptors.[73] Recently, total synthesis of this toxin has been reported.[74]

For several toxins isolated lately from shellfish, it has not been determined whether they are synthesized by microalgae or bacteria and accumlated by filter-feeding molluscs or these substances are endogenous metabolites. Thus poisonings by edible mollusc *Atrina muricata* (*Pinna pectinata*) are occasionally registered in China and Japan. In Japan, 2766 people suffered from such poisoinings from 1975 to 1991. Macrocyclic polyether compounds referred to as pinnatoxins were identified as the toxic agents.[75] Their structure resembles those of azaspiracid and pectenotoxins, they activate calcium channels and seem to be of microalgal origin. The accompanying alkaloid pinnamine (**24**) also brings about toxic symptoms, and its LD_{50} is ~500 μg kg^{-1} (see Ref. 76).



24

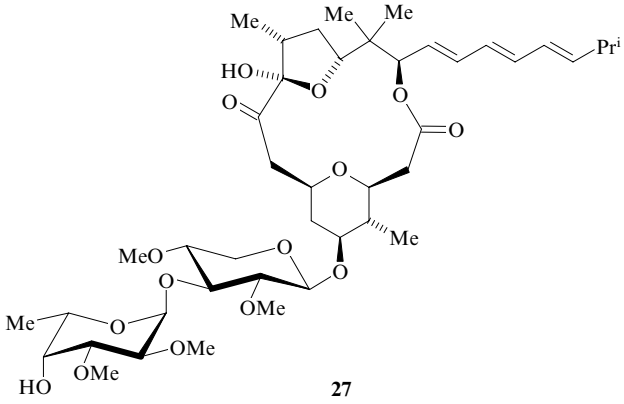
Toxic viscera of another shellfish, the gastropod *Turbo marmorata*, are commonly removed before use. Therefrom, two diiodotyramine derivatives, *viz*., turbotoxins A (**25**) and B (**26**) were isolated. These compounds inhibit acetylcholinesterase and exhibit acute toxicity with LD_{50} of 1 and 4 mg kg^{-1}, respectively.[77]



25, **26**

25: R = Me; **26**: R = H.

Instances of severe poisoning with red algae of the genus *Gracilaria* and related species are known. Thus in 1991 some inhabitants of Guam got severe poisonings when eating the alga *Polycavernosa tsudai*, and for several people it resulted in the fatal outcome. The toxic metabolite proved to be a glycosylated macrolide called polycavernoside A (**27**).



27

Compound **27** is a 13-membered lactone with a triene side chain and glycosylated at the hydroxyl group with a disaccharide moiety comprising methylated D-xylose and L-fucose.[78] The absolute stereochemistry of polycavernoside A was elucidated after its synthesis in 1998 by Japanese scientists from Hokkaido and Tohoku universities.[79] In other cases, poisonous compounds prostaglandin E2 and aplysiatoxin macrolides typical of cyanobacteria contributed to the poisoning with the alga of the genus *Gracilaria*.[80]

III. Marine toxins: isolation and structure determination

Many marine toxins are contained in organisms that biosynthesize or accumulate them in minor amounts.[81] In order to demonstrate the complexity of the isolation of such compounds, let us cite some data on the sequence of procedures required for the isolation of maitotoxin (**18**) needed for structural studies. This toxin was obtained from the cultured dinoflagellate *Gambierdiscus toxicus* (the culture of the species was established from the samples collected from macroalgae growing at the coast of the Gambier Islands, French Polynesia). Four thousand litres of the culture, which contained roughly 2 billion cells of the microalga per 1000 litres of the culture, were grown over a period of one year using three-litre Fernbach flasks. The isolation itself included the following steps:

1) collection of the algal cells from the culture medium by filtration,

2) extraction of the mass with methanol and aqueous methanol,

3) concentration of the extracts *in vacuo*,

4) removal of lipids with dichloromethane,

5) extraction of polar fraction with butyl alcohol,

6) concentration of the butanol extract *in vacuo*,

7) normal-phase chromatography on a silica gel column,

8) reversed-phase chromatography on an ODS-phase column,

9) reversed-phase chromatography on a Develosil C8 column,

10) final purification of the obtained highly toxic fraction by repeated HPLC on a Develosil TMS-5 column.

As a result, 4 – 5 mg of the pure toxin was obtained from 1000 litres of the culture.[53]

Unusal and complex structures of many marine toxins as well as a wealth of chiral centres in them make the determination of their structures a complicated problem. This requires not only the application of state-of-the-art chemical and physicochemical research methods, but also the development of a whole series of new approaches to structural analysis.[1]

Thus when establishing the chemical structure of palytoxin (**17**) with abundant hydroxyl groups (mostly as part of vicinal triols and diols) and bearing four *trans*- and three *cis*-double bonds and many chiral carbon atoms, a whole series of demanding problems including the determination of the absolute configurations of all the stereocentres should be solved. The structure of palytoxin was elucidated by three groups of first-class scientists: a US group headed by professor Moore from the University of Hawaii and Japanese groups (from several Japanese universities) headed by professors Hirata and Uemura. In addition, American synthetic chemists headed by professor Kishi, Woodward's school of the Harvard University, took a very active part in the work.

Neither compound **17** itself, nor its derivatives, despite perennial attempts, could be obtained in a crystalline state in order to apply X-ray diffraction analysis to the determination of their structures. Therefore, a strategy should have been found to degrade it to a comparatively small number of large fragments. Having established the constitution of these fragments, and then having analyzed and assembled the information obtained, one might reproduce the structure of the starting compound.

One of non-standard approaches to the degradation was the partial periodate oxidation of the toxin applied onto a hydrophobic sorbent. In this case, only those diol, triol and polyol fragments that have not been shielded as a result of sorption, rather than all of them, underwent cleavage (otherwise, it would have resulted in too many low-molecular products).

Another approach was the ozonolysis of palytoxin or its derivatives. Around ten major degradation products of compound **17** were isolated and subjected to most thorough structural analysis. They were studied using spin-decoupling NMR spectroscopy and the latest innovations in mass spectrometry for that time. However, the exploitation of only physicochemical methods was not enough, and the researcher had to solve a host of emerging problems with the aid of the chemical transformations of the products obtained.

For example, a Japanese group that reported on a full elucidation of the palytoxin structure in 1981, however, did not include data on the stereochemistry of the majority of chiral centres. In the final step, this research group determined the structure of fragment **28** (Scheme 1).[41]

Compound **28** was prepared from the *N*-*p*-bromobenzoyl derivative of palytoxin by ozonolysis followed by borohydride reduction. It represented a mixture of two epimeric polyols with a molecular weight of 1046 Da [a peak with m/z 1069 $(M + Na)^+$ in a field-desorption mass spectrum (FDMS)]. The ^{13}C NMR spectrum of compound **28** contained a signal of a hemiacetal carbon atom at δ 101.9 ppm. However, upon acetylation this signal disappeared, and the spectrum displayed signals that are characteristic of an unsaturated ketone. The acetylation product corresponded to formula **29**. When treated with 50% acetic acid, compound **28** produced spiroketal **30**. It was impossible to analyze all the signals in the NMR spectra of these compounds due to the complexity of their structures and the fact that they were mixtures of epimers. Finally, periodate oxidation of compound **28** followed by borohydride reduction resulted in more structurally simple fragments, *viz*., compounds **31** and **32** already studied by that time. It is the analysis of their NMR spectra and the spectra of other compounds of this series that allowed determination of the polyol **28** structure.

The solution of stereochemical problems in establishing the full structure of palytoxin (**17**) and other complex marine toxins was particularly challenging. In his recent paper 'Palytoxin: an inexhaustible source of inspiration — personal perspective',[82] Professor Kishi wrote: Our research plan for the palytoxin project is summarized as follows:

1. Synthesize all the possible stereoisomers for a degradation product of palytoxin from a chiral starting material with known absolute configuration.

2. Confirm that all the stereoisomers can be distinguished by spectroscopic and/or chromatographic methods.

3. Find which stereoisomer matches the degradation product.

4. Repeat the same procedure for other degradation products.

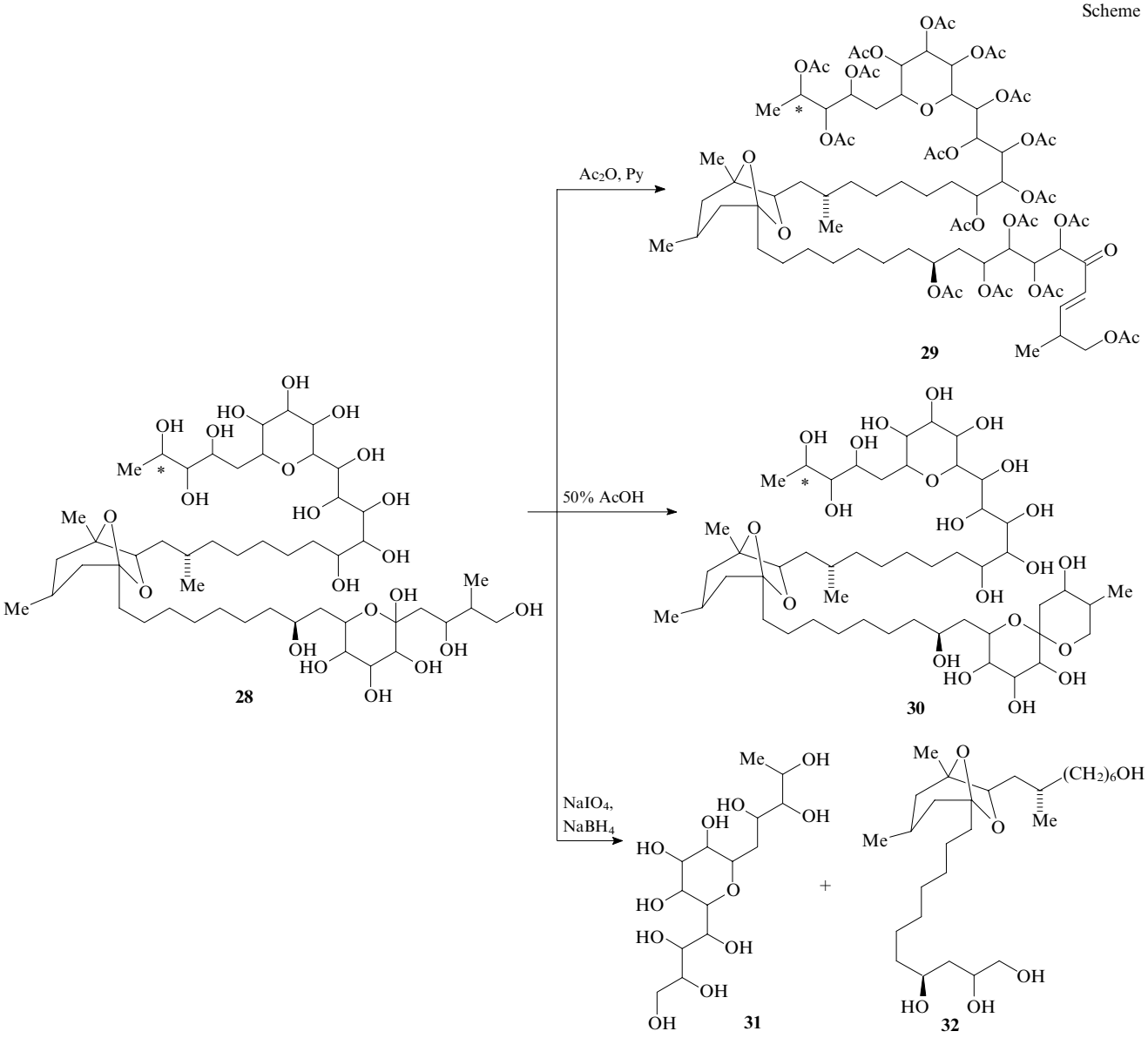
5. Establish the complete structure of palytoxin, by combining all this information.

This plan was implemented, and the complete structure of palytoxin was elucidated with the aid of synthetic chemists, particularly, in the step of absolute configuration determination.

The determination of the structure of maitotoxin (**18**), one of the most structurally complex low-molecular-weight secondary metabolites, became no less significant than the elucidation of the palytoxin structure. The molecular formula of the toxin ($C_{164}H_{256}O_{68}S_2Na_2$) was determined by elemental analysis, mass spectrometry, NMR spectroscopy and analysis of spectral information on fragment compounds obtained. The presence of two sulfate groups in maitotoxin followed from a comparison of m/z values for molecular ion peaks in the fast atom bombardment mass spectra (FAB MS) of the toxin itself and its desulfated derivative.

As in the case of palytoxin, oxidative degradation was carried out in order to determine the chemical structure of maitotoxin. Periodate oxidation was used followed by borohydride reduction. A total of six fragments containing vicinal OH-groups [C(16) – C(17), C(36) – C(37),

Scheme 1



C(53)−C(54), C(57)−C(58), C(69)−C(70) and C(135)−C(136)] underwent cleavage. One of the diol fragments cleaved [C(36)−C(37), indicated with an arrow in formula **18**] was located between the blocks GHIJK and ABCDEF, which allowed obtaining two large fragments of the starting molecule with molecular weights 964 (fragment **A**) and 2328 Da (fragment **B**). In addition, cleavage of the polyol at the C(135)−C(136) bond gave fragment **C**, which, however, was not isolated due to its low molecular weight.[53]

The fragments **A** and **B** were purified and subjected to a thorough examination by using the newest NMR techniques for that time, such as COSY (COrrelation SpectroscopY), DQF-COSY (Double Quantum Filtered COrrelation Spec-

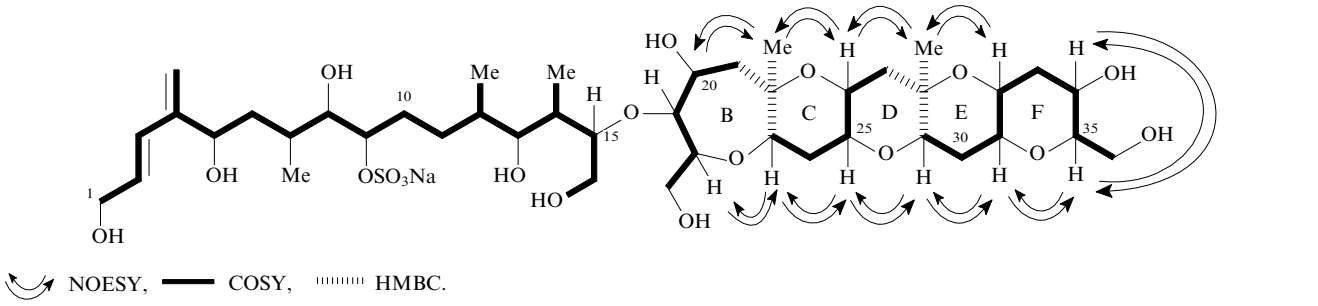


Figure 1. Some key NMR correlations of fragment A derived from maitotoxin.

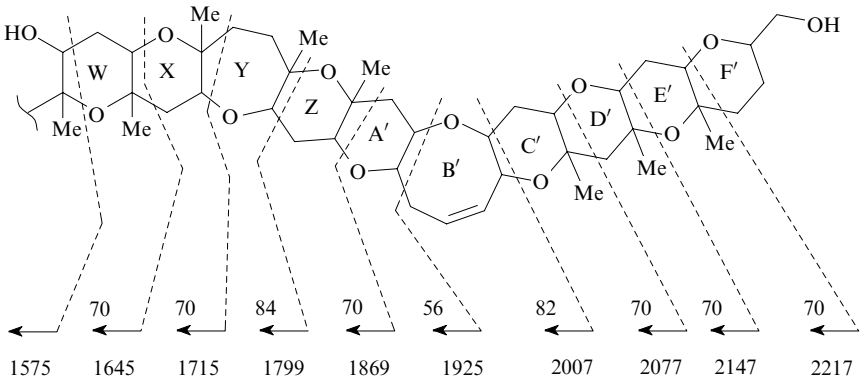


Figure 2. Fragmentation of compound **B** of maitotoxin under conditions of (−)-FAB CAD.

troscopY), TOCSY (TOtal COrrelation SpectroscopY), NOESY (Nuclear Overhauser Effect SpectroscopY and exchange spectroscopy), HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Coherence). Based on the data obtained, the structures of both fragments were determined. Figure 1 shows principal results obtained by applying NMR spectroscopy to the determination of the stucture of fragment **A** of maitotoxin (**18**), which proved planar.

The structures of both fragments gained support when studying their Fast Atom Bombardment Collison-Activated Dissociation mass spectra [(−)-FAB CAD MS/MS]. This method enables the fragmentaion of pseudomolecular ions to be traced under fast atom bombardment conditions in a collision chamber. This method proved quite informative. The spectra contained ion peaks resulting from the cleavage of each of the numerous rings in periodate oxidation products obtained from maitotoxin. This is exemplified by the fragmentation of one of the termini of compound **B** (Fig. 2).[53]

Thus, the investigation of maitotoxin using two-dimensional NMR spectroscopy and CAD MS/MS as well as the analysis of the structures of its oxidative degradation products resulted in the determination of the structure and, partially, stereochemistry of this toxin.[53] The relative and absolute configurations of all the chiral centres in maitotoxin were elucidated in the mid-1990s by research groups headed by Yasumoto,[83] Tachibana [84, 85] and Kishi.[86] Recent doubts about the stereochemistry of the fusion of rings J and K in maitotoxin inferred on the basis of a hypothesis about the biosynthesis of this toxin,[87] have not been supported in the studies by Nicolaou.[88]

The following methods of the stereochemical analysis of complex marine toxins were used most widely:

1) determination of spin-spin coulpling constants and nuclear Overhauser effects for protons located at the sites of ring fusion;

2) calculation of these and other experimental parameters by molecular mechanics methods and their comparison with experimental data;

3) synthesis of chiral model compounds including some rings or other, or synthesis of fragments of this toxin and a comparison of their ^{1}H and ^{13}C NMR spectra with those of the native toxin;

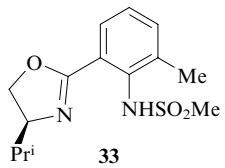
4) determination of the absolute configuration of selected chiral centres by preparing derivatives with chiral agents [methoxyphenylacetyl chloride or 2-methoxy-2-phenyl-2-(trifluoromethyl)acetyl chloride, *etc.*], followed by analysis of their NMR spectra.[89]

IV. On the syntheses of marine toxins

Extensive material on the total syntheses of marine toxins was reviewed in detail elsewhere;[44, 90–94] hence, in the present work we will address only several state-of-the-art synthetic approaches and reactions that have repeatedly been employed in the steps preceding these syntheses and the proper syntheses. This kind of reactions are usually carried out under mild conditions, they feature high efficiency and, undoubtedly, will find wider application in future.

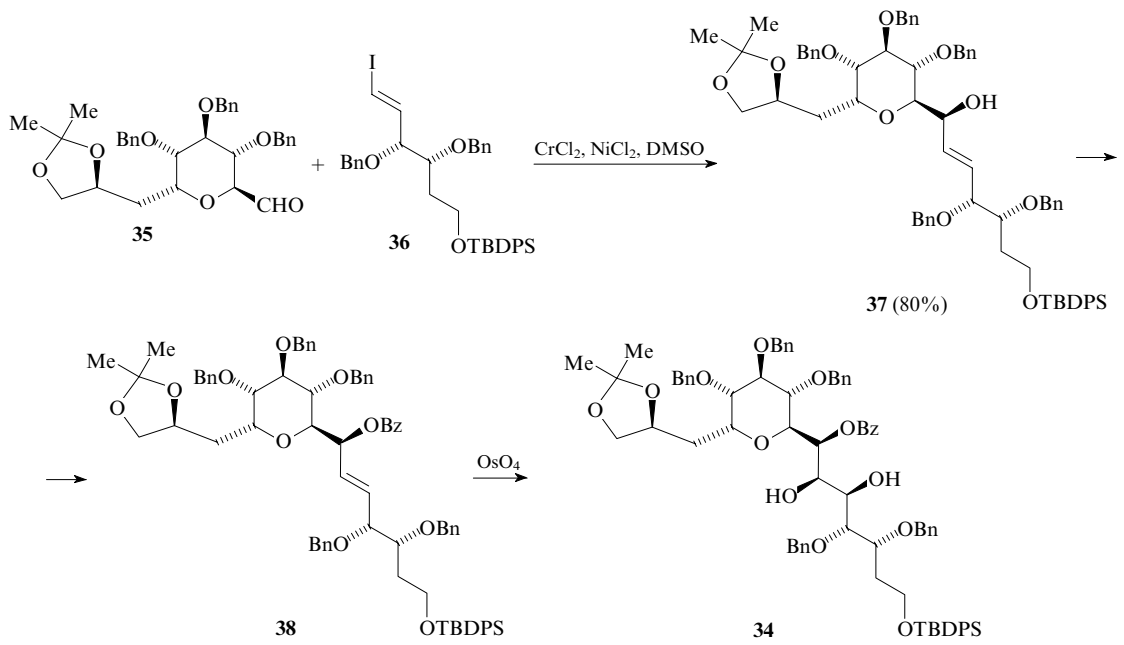
Mild methods of coupling of two compounds to form a new covalent bond played a major role in the realization of syntheses of marine toxins bearing various functional groups and numerous chiral centres. Apart from the long-known Wittig reaction, currently, a number of other approaches are frequently used for this purpose.

Thus in the synthesis of palytoxin carboxylic acid, Kishi [44, 82] exploited a coupling reaction of an alkenyl halide with an aldehyde catalyzed by Ni(II) and Cr(II) salts resulting in a C−C bond. The reaction occurs in dimethyl sulfoxide in the presence of $CrCl_2$ and trace amounts of $NiCl_2$, being an updated version of the previously known coupling reaction of alkenyl halides with aldehydes, which takes place in the presence of $CrCl_2$. The use of traces of $NiCl_2$ considerably increases the efficiency of the process. Although the formation of the C−C bond can also be accomplished with the use of Grignard reagents, organolithium- or organocopper compounds, the coupling procedure developed by Kishi *et al.*[44, 82] offers a number of advantages. Firstly, it is applicable also to polyfunctional substrates with miscellanious protections of the chemically reactive groups and, thus, it can be employed in the last steps of multistep tranformations. Secondly, the stereoselectivity of the reaction can be increased by the introduction of a chiral ligand **33**.



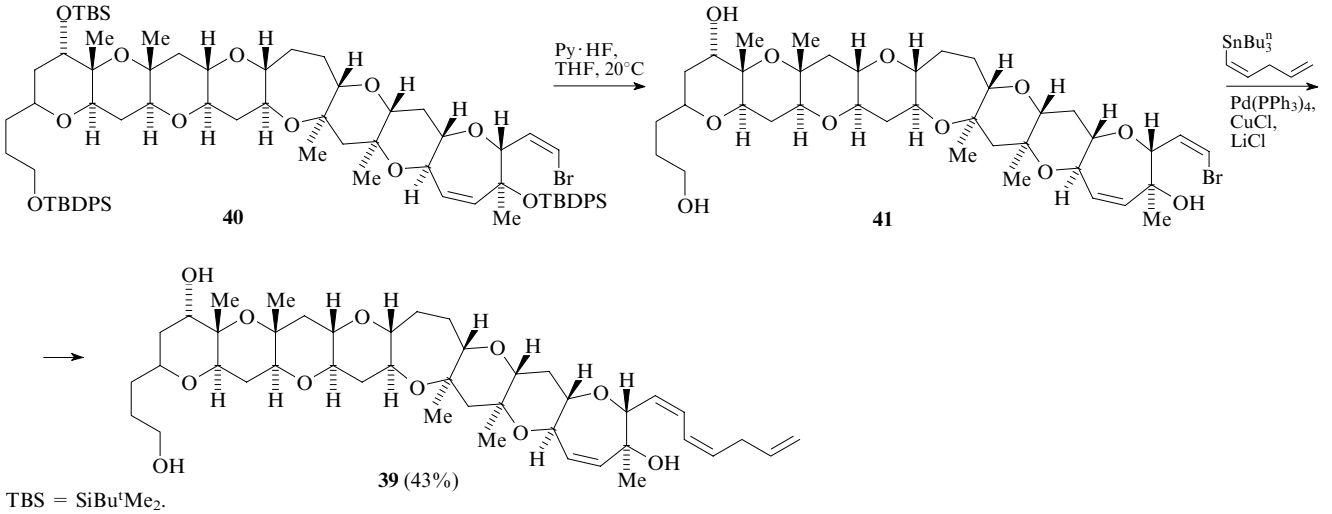
33

Scheme 2



TBDPS = $SiPh_2Bu^t$.

Scheme 3



TBS = $SiBu^tMe_2$.

This improved coupling technique is referred to as the Nozaki–Hiyama–Kishi reaction. Below it is exemplified with the synthesis of the C(8)–C(22) fragment of palytoxin (compound **34**) from aldehyde **35** and alkenyl iodide **36**. Product **37** was converted to benzoate **38**, followed by stereoselective oxidation to compound **34** by osmium(IV) oxide (Scheme 2).[82]

Synthesis of palytoxin carboxylic acid itself was performed using eight fragments accessible in preparative amounts from synthetic chemists of the Harvard University; the assembly of these fragments by coupling reactions established the C(1)–C(7) and C(8)–C(51) bonds and the double bond C(98)=C(99).[43, 44, 82]

Various versions of palladium-catalyzed coupling have been widely used in the syntheses of marine toxins. The general scheme of reactions of this type is as follows:

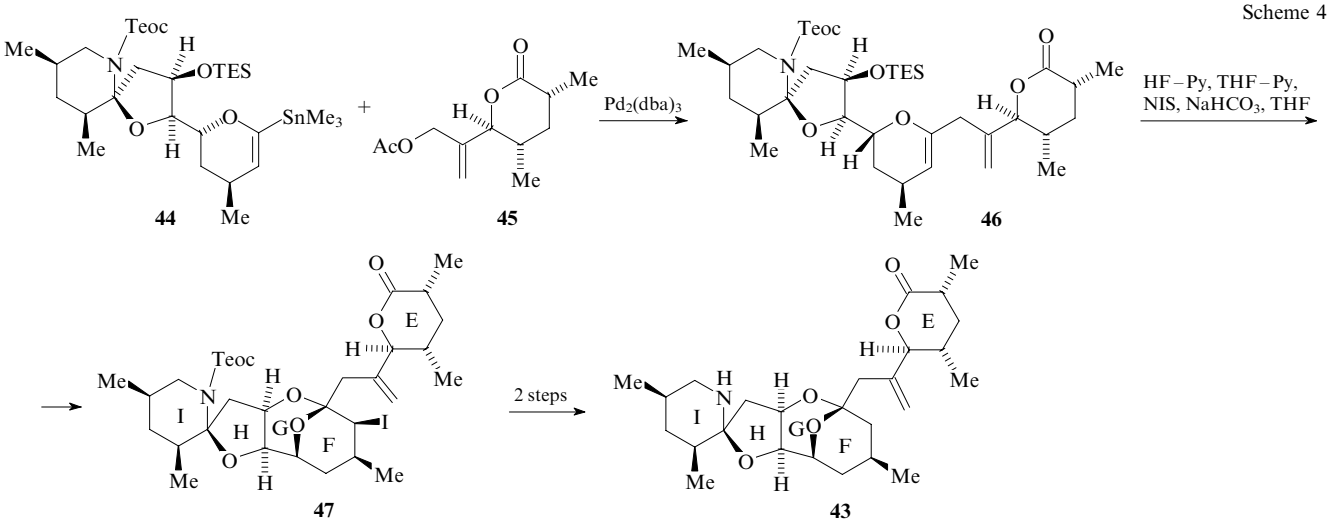
$$R^1{-}M + R^2{-}X \xrightarrow{\text{Pd catalyst}} R^1{-}R^2 + MX$$

R^1M is an organometallic reagent, X is a halogen or the triflate group.

Sometimes, other functional groups can also serve as the substitutent X, and palladium phosphine complexes [for example, $Pd^0(PPh_3)_4$ or $PdCl_2(PPh_3)_2$], palladium complex with dibenzylideneacetone, *etc.* can be employed as the catalysts.

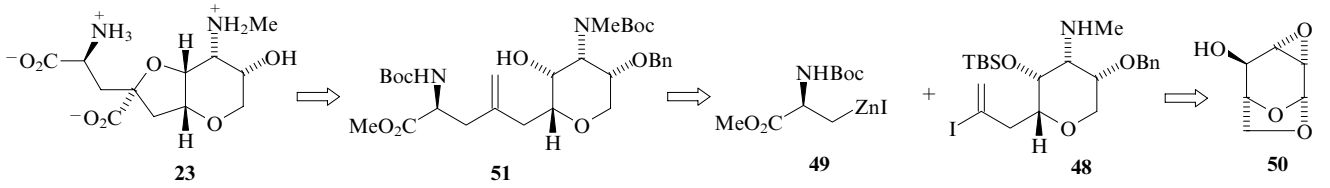
The coupling reaction involving organotin compounds is referred to as the Stille reaction. It is often exploited in the syntheses of marine toxins. For instance, in a total synthesis of gambierol (**39**),[95] a highly toxic compound from *Gambierdiscus toxicus*, which is related to ciguatoxin, this approach was used for the construction of the toxin side chain containing a *cis*,*cis*-diene. Scheme 3 illustrates the use of

Scheme 4



TES = $SiEt_3$, Teoc = $C(O)O(CH_2)_2SiMe_3$, dba is dibenzylideneacetone, NIS is *N*-iodosuccinimide.

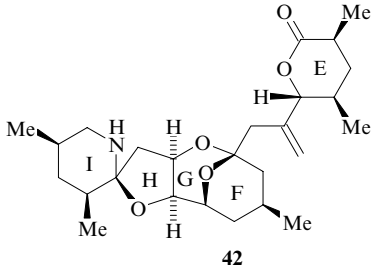
Scheme 5



Boc = CO_2Bu^t.

the Stille reaction in the synthesis of gambierol. First, compound **40** was desilylated, then bromide **41** reacted with the organotin compound. The efficiency of this conversion was increased by addition of CuCl and LiCl to a palladium catalyst (the Corey modification).

Nicolaou *et al.*[96,97] have recently applied similar coupling to the synthesis of azaspiracid-1 (**13**). Thus fragment **42** of azaspiracid-1 obtained by esterification of the carboxyl group of this toxin followed by periodate oxidation contained rings I, H, G, F and E.



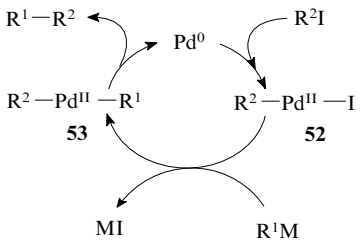
42

The absolute stereochemistry of the fragment **42** was established following synthesis of compound **43** with epimeric chiral centres in ring E (Scheme 4). As a result, the structure of azaspiracid-1 suggested earlier was revised. The key step in the synthesis of polycycle **43** was coupling of two precursors, compound **44** containing rings I, H, F, and compound **45** containing ring E. This resulted in compound **46** further transformations of which led to construction of ring G yielding product **47**. The latter was converted to the target product **43**.

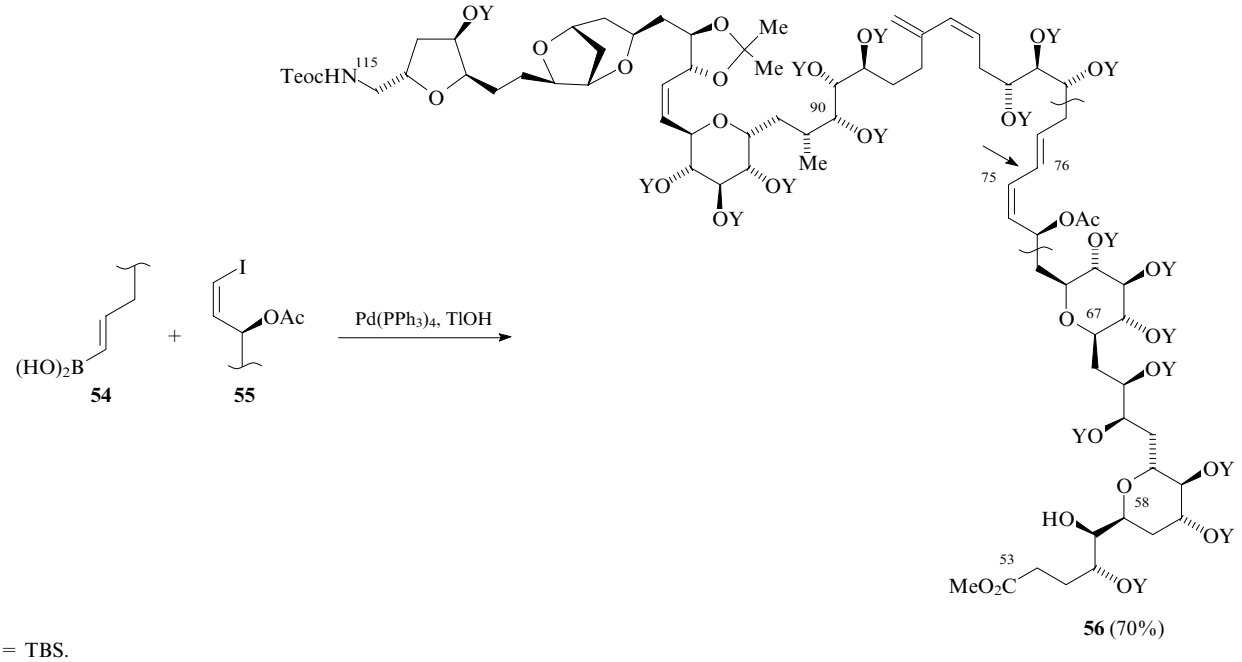
A similar reaction, with exception of being based on the use of an organozinc derivative (the Negishi reaction and its modifications), has recently been employed in the synthesis of dysiherbaine (**23**).[73] As followed from the retrosynthetic analysis of structure **23** shown in Scheme 5, the key step could be a reaction of halide **48** with derivative **49** to form intermediate **51**. Dianhydrohexose **50** was suggested to serve as a precursor of halide **48**. The synthesis plan was completely implemented, and the reaction **48** + **49** → **51** proceeded with a high product yield using $PdCl_2(PPh_3)_2$ as the catalyst.

In the synthesis of palytoxin (**17**), Kishi[44] used an improved coupling of an organoboron derivative with an alkenyl iodide (the Suzuki–Miyaura[98] reaction). One of the basic problems was the assembly of two fragments to form the C(75)–C(76) bond. Palytoxin itself is insoluble in organic solvents, therefore in the final synthetic steps, mixtures of a solvent and water were employed. This coupling technique offers an important advantage over other methods because it can be performed in the presence of water. The coupling of two starting compounds, aimed at the construction of a *trans–cis* diene system in the product formed, proceeds smoothly for relatively small model substrates, however, an increase in molecule sizes resulted in a sharp drop in the yields of the desired products. The

Scheme 6



Scheme 7



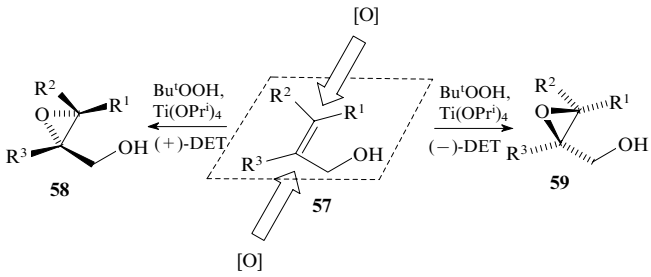
Y = TBS.

solution of this problem required that the mechanism of this reaction in the presence of Pd(0) catalysts be examined (Scheme 6). It was established that the oxidative addition of vinyl iodide to palladium occurs in the first step to form divalent palladium complex **52**. Afterwards, this complex reacts with an alkenyl borate to replace the halogen atom with a fragment of the organoboron reactant resulting in a new complex **53**. Reductive elimination of the reaction product from complex **53** leads to the regeneration of the palladium catalyst.

Kishi [44] established that the addition of TlOH to the reaction mixture sharply increases the reaction efficiency since the replacement of the anion in complex **52** yields TlI, which is insoluble in the reaction mixture. This improvement permitted the required coupling reaction of substrates **54** and **55** to be carried out to form the C(53)–C(115) fragment (left portion) of palytoxin (**56**) in high yield (Scheme 7).

An essential role in the synthesis of marine toxins was played by the development of efficient methods for the preparation of tetrahydropyran and other oxygen-containing rings that are abundant in many polyether-type marine toxins. For the synthesis of cyclic ethers, several approaches have been developed. One of them is the cyclization of hydroxyalkyl vinyl epoxide. The asymmetric epoxidation of unsaturated alcohols, which is suitable for the preparation of such epoxides, has been developed not long before at Stanford University, and it is known now as the Sharpless epoxidation.[99] The epoxidation takes place on treatment of the corresponding allylic alcohol **57** with diethyl (+)- or (−)-tartrate, titanium tetraisopropoxide and *tert*-butyl hydroperoxide in dichloromethane at low temperature. The use of enantiomeric tartrates results in an attack by an oxygen atom on the double bond plane from the opposite sides, and, consequently, in different stereoisomeric epoxides (**58** or **59**) (Scheme 8).

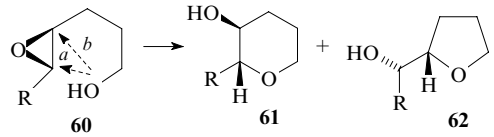
Scheme 8



DET is diethyl tartrate.

6-*endo*-Cyclization of hydroxyalkyl vinyl epoxides developed by Nicolaou [100] was studied on model compounds using 10-camphorsulfonic acid (CSA) as the catalyst in dichloromethane. It was established that the direction of cyclization (the predominant formation of 6-*endo*- or 5-*exo*-products) of compounds of the general formula **60** depends on the substituent R. If R contains a vinyl group, only 6-*endo*-product **61** is produced, whereas 5-*exo*-product **62** is formed in the absence of a multiple bond in this substituent (Scheme 9).

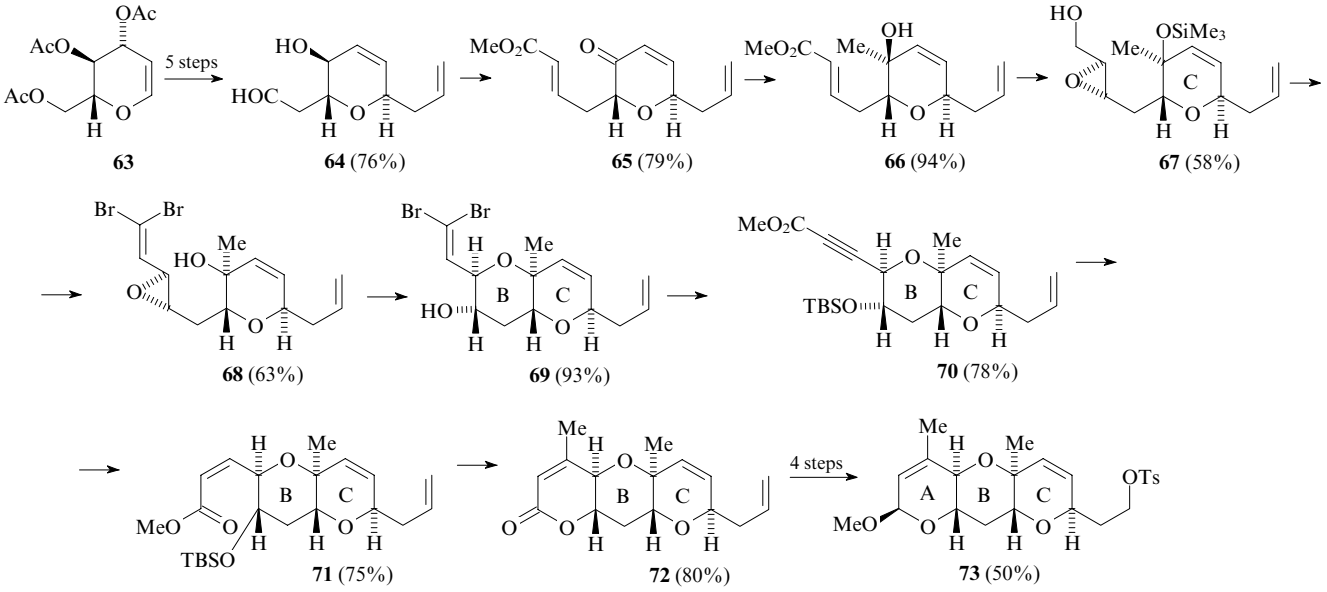
Scheme 9



(*a*) 6-*endo*-cyclization; (*b*) 5-*exo*-cyclization.

R	**61** : **62**
$CH_2CH_2CO_2Me$	0 : 100
$CH{=}CH_2$	100 : 0
$CH{=}CHCO_2Me$	60 : 40
$CH{=}CBr_2$	100 : 0

Scheme 10



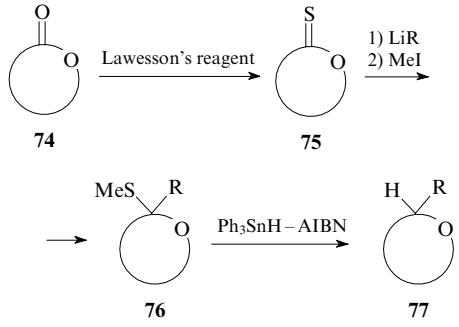
The vinyl substituent can relatively easily be introduced into the starting compound, subsequent modification prearranges the product for ensuing 6-*endo*-cyclization in the synthesis of polycyclic fragments of the polyether toxins. Thus, a general approach was found to solve the problem of the preparation of marine toxin fragments comprising several tetrahydropyran rings.

This approach was applied to the asymmetric synthesis of a fragment of brevetoxin B (**6**) containing rings A, B and C (Scheme 10).[100] The starting compound used was triacetyl-D-glucal (**63**) from which derivative **64** was obtained by treatment with allyltrimethylsilane and $TiCl_4$, then with potassium carbonate in methanol followed by conversion of the hydroxymethyl group to the aldehyde group.

The Wittig reaction of compound **64** with $Ph_3P{=}CHCO_2Me$ followed by mild Swern oxidation [oxidation of alcohols to carbonyl compounds upon treatment with the system $Me_2SO-(COCl)_2$ (Ref. 101)] and the reaction of intermediate unsaturated ketone **65** with $AlMe_3$ resulted (predominantly) in alcohol **66** required for further transformations. This was converted to derivative **67** containing ring C of the target fragment by silylation, reduction of the ester group and asymmetric Sharpless epoxidation. The subsequent Swern oxidation and the Wittig reaction followed by desilylation resulted in compound **68** suitable for 6-*endo*-cyclization to form ring B. The obtained derivative **69** contained already two out of three required rings. Its silylation with *tert*-butyldimethylchlorosilane followed by reaction with an excess of butyllithium and MeOCOCl yielded acetylenic ester **70**. This was treated with methylmagnesium chloride and CuI at low temperature to give unsaturated ester **71**, which on subsequent treatment with tetrabutylammonium fluoride cyclized to compound **72** containing rings A, B and C of brevetoxin. After a series of transformations in ring A and the side chain, the target derivative **73** was obtained from compound **72**. Compound **73** was then used in a total synthesis of brevetoxin **6**.[102]

Another synthetic method to cyclic ethers proposed by Nicolaou *et al.*[103] was the nucleophilic addiion of organolithium compounds to thiolactones followed by reduction of the adducts obtained. The starting compounds in this case were accessible lactones of the general formula **74** including macrocyclic ones. It should be noted that lactones cannot directly be used in the nucleophilic addition reactions because of instability of the products (their tendency to undergo ring-opening), hence they were first converted to thiolactones upon treatment with Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide prepared from anisole on heating in P_2S_5 (Ref. 104)]. Nicolaou *et al.*[103] demonstrated that thiolactones with seven-to-seventeen-membered rings of the general formula **75** reacted with various organolithium compounds in THF (−78 °C) being subsequently quenched with methyl iodide to give adducts **76** in nearly quantitative yields. Adducts **76** were reduced with Ph_3SnH in the presence of AIBN as the catalyst on heating in toluene. The overall yields of the products, substituted cyclic ethers **77**, were 70%–80% (Scheme 11).

Scheme 11



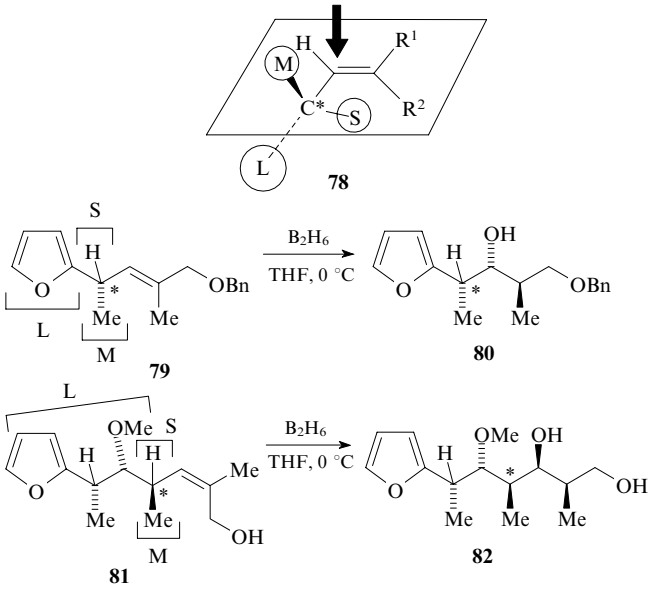
R = Alk, $CH_2{=}CH$ *etc.*; AIBN is 2,2′-azobis(isobutyronitrile).

This method of the formation of oxygen-containing rings was employed in the preparation of many polyether marine toxins.

Of special significance in the synthesis of marine toxins bearing a large number of stereogenic centres was the development of directed stereospecific methods for the introduction of a hydroxyl group into a substrate already containing such centres. These methods include, for example, hydroboration. The stereochemical features of the hydroboration of chiral substrates had been studied by Kishi *et al.*[44] before he employed this method for the elucidation of the palytoxin structure. They stem from the

fact that in most cases the predominant conformation of unsaturated compounds with a chiral centre adjacent to a double bond is conformation **78** (Scheme 12).

Scheme 12



In other words, the rotamer that has the smallest substituent (S) coplanar with the double bond at the chiral carbon atom is most stable (because of steric interaction with the substitunent R^2, this is more favourable than the presence of larger substituents at this site). Therefore, the approach of a reactant to a double bond will be less hindered from the side of a medium-sized substituent (M) than from the side of a larger substituent (L). This governs the results of hydroboration and allows the stereospecific introduction of a hydroxyl group to the position adjacent to the chiral centre. For example, compound **79** yields predominantly isomer **80** upon hydroboration, while compound **81** gives isomer **82** with the opposite configuration of the chiral carbon atom formed as a result of the reaction.

Many of the above-mentioned reactions were employed in the syntheses of hemibrevetoxin B (**83**), one of the simplest polyether toxins, and played an important role in the development of the total syntheses of structurally more complex marine toxins. The first synthesis of hemibrevetoxin B was reported by Nicolaou *et al.*[105, 106] 3 years after its isolation.[107] A review by Nakata[90] summarizes data on numerous approaches to the preparation of this compound published before 2005. Each approach is described as a sequence of synthetic methods applied to the construction of the four rings and side chain of this toxin.

The Nicolaou synthesis was carried out according to a linear rather than convergent strategy. In this approach, ring A resulted from D-mannose pentaacetate (**84**), which underwent initially the Lewis acid-catalyzed allylation (the reaction sequence **84** → **87**). Ring B was constructed with involvement of *endo*-cyclization of the corresponding vinyl epoxide (**88** → **89**). Ring C was built by sequential thiolactonization, alkylation and hydroboration (**89** → **91**). Ring D was formed analogously, *i.e.*, involving thiolactonization, alkylation and introduction of a methyl group with a Grignard reagent (**91** → **94**). Finally, the side chain was synthesized by the Wittig reaction followed by methylenation (**94** → **95** → **83**). The reaction sequence employed for the total synthesis of hemibrevetoxin B (**83**) is briefly depicted in Scheme 13.

Table 1. The use of some reactions in the synthesis of marine toxins.

Reaction	Toxins	Ref.
The Nozaki – Hiyama – Kishi reaction	palytoxin	43, 44, 82
	pinnatoxin A	110
	brevetoxin B	90
The Stille reaction	azaspiracid-1	96, 97
	(−)-brevenal [a]	111
	amphidinolide	112
	gambierol	95, 113
	16-epi-gambierol	114
The Suzuki – Miyaura reaction	palytoxin	44
	brevenal	110
	gymnocin-A	90
	gambierol	109, 113
The Negishi reaction and its modifications	dysiherbaine	73
	hemibrevetoxin	90
	amphidinolide	112
The Sharpless epoxidation	brevetoxin B	93
	hemibrevetoxin	90
	gambierol	90
6-*endo*- and 7-*endo*-cyclization of hydroxy vinyl epoxides	hemibrevetoxin	90, 105, 106
	brevetoxin B	90, 93
	brevetoxin A	90, 93
	gambierol	90, 93
Synthesis of oxygen-containing heterocycles *via* thiolactones	hemibrevetoxin	90, 105, 106
	brevetoxin A	90
Hydroboration	palytoxin	43, 44
	gymnocin A	90, 93
	brevetoxin A	90
	gambierol	113

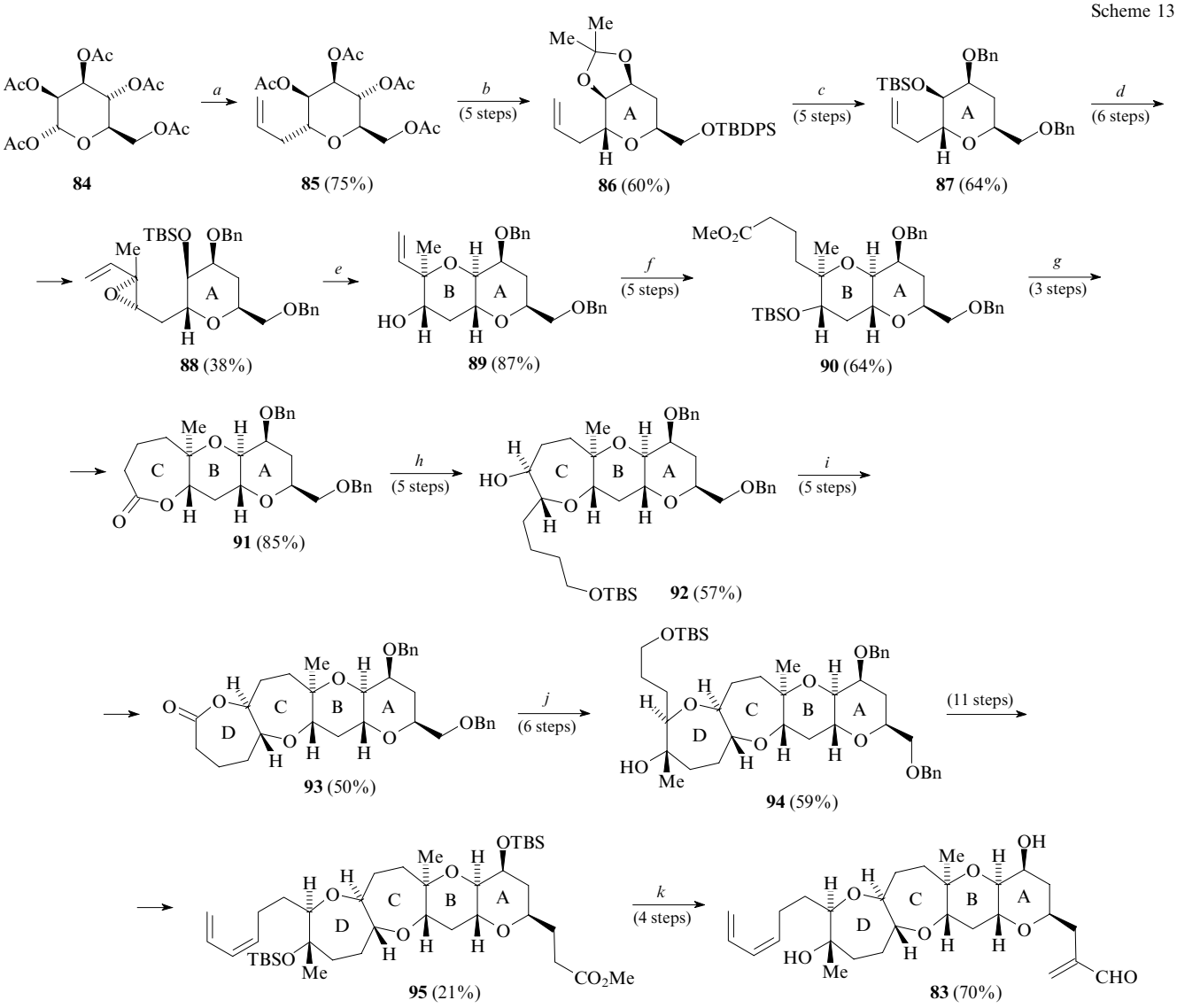
[a] Cu(I) - thiophene-2-carboxylate modification of the reaction.

The use of these and other apporaches to the synthesis of diverse marine toxins was discussed in recent reviews by Japanese chemists.[108, 109] The majority of the syntheses were conducted according to convergent strategies by coupling of pre-formed building blocks. Table 1 lists examples of the use of some reactions addressed in this paper in marine toxin syntheses, mainly accomplished already in the 21st century.

V. Detection and quantitation of marine toxins

Intoxications associated with the consumption of some marine species, especially, shellfish and fish, cause health damage to inhabitants of many countries. The toxicity of seafood also results in economic losses because of partially rejected marine harvests. The rapid development of mariculture farms all over the world and increase in fish and other seafood production in tropical waters make the problem of marine toxins from year to year more and more vital. The solution of this problem is impossible without the development of highly sensitive and specific methods of analysis of marine toxins. These methods have been continuously upgraded over many years, and their

Scheme 13



(*a*) $CH_2{=}CHCH_2TMS$, $BF_3 \cdot Et_2O$, TMSOTf; (*b*) 1) MeONa, 2) TBDPSCl, Im, 3) CSA, $CH_2{=}C(OMe)Me$, 4) $Im_2C{=}S$, 5) Bu^n_3SnH, AIBN; (*c*) 1) TBAF, 2) NaH, BnBr, 3) TFA, 4) Bu^n_2SnO, BnBr, CsF, 5) TBSOTf, 2,6-lutidine; (*d*) 1) O_3, CH_2Cl_2, Ph_3P, 2) $Ph_3P{=}C(Me)CO_2Me$, 3) DIBAH, 4) Sharpless AE, 5) SO_3-Py, 6) $Ph_3P{=}CH_2$; (*e*) 1) TBAF, 2) CSA; (*f*) 1) TBSOTf, 2) BF_3-THF, H_2O_2, 3) Swern ox, 4) $Ph_3P{=}CHCO_2Me$, 5) H_2, Pd/C; (*g*) 1) LiOH, THF, 2) TBAF, 3) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, DMAP, PhH; (*h*) the Lawesson reagent, 2) $TBSO(CH_2)_4$(2-Th)$Cu(CN)Li_2$, 3) LiHMDS, $PhNTf_2$, HMPA, 4) $BH_3 \cdot THF$, 5) H_2O_2, NaOH; (*i*) 1) Ac_2O, 2) CSA, $MeOH-CH_2Cl_2$, 3) PDC, DMF, 4) K_2CO_3, MeOH, 5) 2,4,6-$Cl_3C_6H_2COCl$, Et_3N, DMAP, PhH; (*j*) 1) the Lawesson reagent, 2) $TBSO(CH_2)_3$(2-Th)$Cu(CN)Li_2$, 3) $BH_3 \cdot THF$, 4) Swern ox, 5) DBU, PhMe, 6) MeMgBr; (*k*) 1) DIBAH, 2) Swern ox, 3) $CH_2{=}NMe_2I$, Et_3N, 4) SiF_4, $MeCN-CH_2Cl_2$; TMS is trimethylsilyl, Tf is trifluoromethylsulfonyl, Im is imidazol-1-yl, TBAF is tetra(n-butyl)ammonium bromide, TFA is trifluoroacetic acid, DIBAH is di(isobutyl)aluminium hydride, Sharpless AE is the Sharpless asymmetric epoxidation, Swern ox is the Swern oxidation of alcohols, DMAP is 4-dimethylaminopyridine, Th is thienyl, HMDS is hexamethyldisilazide, HMPA is hexamethylphosphoramide, DBU is 1,8-diazabicyclo[5.4.0]undecane.

development exerted a considerable influence on the progress in the methods of analytical chemistry.

1. Analysis methods that rely on biological test systems

The presence of toxins is often detected by testing biomaterial for acute toxicity on mice (the so-called MBA-Test). The advantage of the method is its exceptional simplicity and a quick answer to the question whether the biological material under analysis represents danger for humans and animals. This method is exploited in many countries. However, despite being straightforward, it is comparatively expensive, associated with serious ethical problems and provides no information on the nature of toxins. Although testing on mice is a standard method of marine toxicology and included in the rules of official organizations, such as the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA), still it finds no general application.

Other biological methods based on, for example, the use of membrane cell receptors and channels are relatively uncommon.[115] By using such assays, one can detect and quantitate only those toxins for which molecular mechanisms of action are known and the corresponding molecular

targets are available. Most often, the analysis is carried out by probing the voltage-dependent sodium current using rat neurons or by selected ion monitoring, SIM.[116–118] These procedures are utilized mainly for the determination of tetrodotoxin or PSP-toxins.

Finally, some toxin groups can be determined by biotests that rely on the measurement of enzyme activity. Thus there are test systems for the measurement of the effect of a sample on protein phosphatases, for instance, commercially available colorimetric protein phosphatase assay (VDM-Test). It is used for the determination of okadaic acid and some other DSP-toxins.[119, 120]

2. Physicochemical methods of analysis

The methods based on chromatography enable quantitative estimation of a toxin content and provide information on the composition of toxin fractions.[121] At the same time, different toxin groups substantially differ in their chemical nature and chromatographic properties, and, consequently, each requires specific chromatorgraphy and detection conditions.

For example, neurotoxins including PSP-toxins are commonly analyzed by liquid chromatography followed by fluorescence detection (LC-FD). To this end, toxins undergo oxidative or alkaline treatment to yield fluorescent derivatives.

In the present-day analysis, both pre-column and post-column toxin derivatizations are used.[122] This method has recently been employed for the determination of the so-called 'toxin profile', *i.e.*, the composition of a toxin fraction of the microalaga *Alexandrium catanella* collected at the coast of Chile.[123] Orlova *et al.*[124] studied the morphogenetic differences and toxicity of a large series of samples of the microalga *A. tamarense* collected at the east coast of Russia. Using HPLC, the authors demonstrated that all the samples regardless of the morphogenetic features of microalgae contained PSP-toxins.

Many toxins do not yield fluorescent products upon oxidation or other chemical transformations. In these cases, fluorescent tags are first introduced followed by analyses using HPLC with a fluorescence detector. For instance, polyether toxins in a sample can be converted to fluorescent derivatives on treatment with 9-anthracenyldiazomethane. Further use of HPLC allows the detection of okadaic acid and other DSP-toxins.[125]

It should be noted that LC-FD and other chromatographic methods sometimes provide false results.[6] Hence, HPLC is frequently used together with mass spectrometry. Such combined analytitical technique (LC-MS) for the analysis of toxic fractions is currently a major one, particularly, in research projects that apply to marine toxins. Establishing not only the retention times of the corresponding toxins, but also their mass spectrometric characteristics during analysis repeatedly increases the reliability of data obtained.

The use of LC-MS expands the geography of research on marine toxins every year. For example, by using this method in studying the activation of sodium channels in the neuroblastoma N2a cells it was demonstrated that ciguatera toxins are present in predatory fish dwelling at the coast of Cameroon (west African coast).[126] Azaspiracids were detected in mussels collected at the coast of Morocco in the Mediterranean Sea.[127] Pectenotoxin-1 was found in the phytoplankton of the North Sea.[128]

The instruments for LC-MS analysis are being continuously developed. Thus four types of mass spectrometers, *viz.*, triple quadrupole (TQ), time-of-flight (TOF), quadrupole time-of-flight (q-TOF) and ion trap (IT), have recently been compared in the analysis of okadaic acid and pectenotoxin-2. All four of the mass spectrometer detectors were found to exhibit a linear dependence of molecular ion peak heights on the concentrations of these toxins and good precision, the TQ-instrument manifesting the highest sensitivity. This allows detection of as small amounts as 12.2 pg of okadaic acid and 2.9 pg of pectenotoxin-2 applied to an LC-MS-column.[129]

Recently, reports have appeared on the successful use of MALDI triple quadrupole mass spectrometry for the detection and quantitation of spirolides[130] and on the use of electrospray ionization mass spectrometry (ESI MS) for the analysis of DSP-toxins and yessotoxins.[131, 132]

Sample pre-treatment techniques are also being perfected. It was proposed to use solid-phase pre-sorption on a special synthetic porous-polymeric material (SPATT bags) to analyze water-soluble marine toxins. Toxic microalgae blooms were found to favour solubility of lipophilic toxins in seawater; pectenotoxins, yessotoxins, okadaic acid analogues and other toxins can be extracted from SPATT and subjected to an LC-MS-analysis.[133] A similar approach using C18-solid-phase sorption was employed for the determination of domoic acid in seawater and phytoplankton.[134] In addition, such sorption can reduce the so-called 'matrix effects', *i.e.*, the influence of interfering substances on peak intensities in mass spectra. The best results were obtained by preliminary sample sorption on polymeric sorbents and chromatographic separation using base-containing solvent systems.[135]

For the purification of extracts derived from shellfish and containing DSP-toxins, it was proposed to remove contaminating substances by gel-permeation chromatography prior to LC-MS-analysis.[136]

However, the most significant development was the use of the LC-MS-MS-analysis for the detection of toxins. Equipment with a tandem mass spectrometic analyzer allows not only identification of characteristic peaks in the mass spectra of compounds under study, but also elucidation of mass spectrometric fragmentation sequences, *i.e.*, the origin of any fragment ion. This results in a reliable identification of the majority of toxins.

This method, for example, permitted the determination of diarrhetic toxins in extracts obtained from only one cell of each of several *Dinophysis* species collected near the Hokkaido island.[137] Such instruments were reported to have been mounted on research vessels, which enables analysis of the biological material shortly after collection, rather than of samples delivered to a specializied laboratory. For example, using LC-MS-MS, phytoplankton samples collected at the coasts of Norway, Scotland and Denmark were analyzed on board; microalgae of the North Sea most often contained domoic acid and 20-methylspirolide G.[138]

Of the physicochemical methods even more sensitive than LC-MS, capillary electrophoresis should be mentioned. It is applicable, above all, to the analysis of toxins bearing readily ionizable groups, for example, domoic acid.[139] In combination with ESI MS, it was recently employed for the determination of yessotoxins and pectenotoxins in molluscs.[140]

3. Immunological methods

Immunological methods including enzyme immunoassay and enzyme-linked immunosorbent assay are also widely exploited for the detection of marine toxins and their quantitation. There are well-developed protocols of such assays, and required reagent kits are commercially available. As is known, enzyme immunoassay relies on the use of antibodies to the corresponding toxins. For the production of antibodies, it is necessary to prepare a synthetic antigen comprising a protein (for example, albumin) with toxin (hapten) molecules covalently attached to it. Immunization of aminals with such an antigen gives rise to the production of antibodies.

However, many marine toxins are available in very small amount, whereas immunization of an animal aimed at obtaining antibodies requires them in comparatively large quantities. Recently, it has been shown that instead of a native toxin, its fragments prepared by organic synthesis can be used. Oguri [141] employed two synthetic compounds, *viz*., those containing rings A, B, C, D, E and rings I, J, K, L, M of ciguatoxin (**19**), respectively, for the detection and quantitative determination of ciguatoxin 3C. Both haptens were attached to a protein, and upon immunization of mice with the obtained antigens monoclonal antibodies to the 'left' and 'right' halves of the toxin were elicited. Then 'right' antibodies were conjugated with peroxidase. Enzyme-linked immunosorbent assay (ELISA) developed based on these antibodies (Fig. 3) was highly sensitive to ciguatoxin with no cross-reactivity against structurally similar toxins including brevetoxins and okadaic acid.

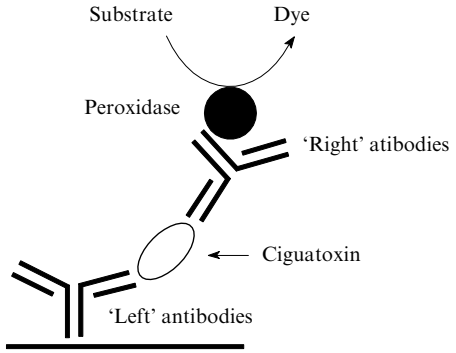


Figure 3. Scheme of the enzyme immunoassay of ciguatoxin.[141]

Development of immunosensors is another trend in the state-of-the-art analysis of marine toxins. In recent years, surface plasmon resonance (SPR) has widely been used for biosensor development. An optical sensor based on SPR was applied to the detection of okadaic acid in molluscs. Monoclonal antibodies to okadaic acid were obtained upon immunization of mice with a synthetic antigen based on thyroglobulin with conjugated okadaic acid. The antibodies were immobilized on a chip surface and made it possible to perform up to 50 analyses per chip. The method was not inferior to LC-MS in terms of sensitivity.[142]

VI. Conclusion

Among marine toxins, compounds were found that are unique in terms of chemical structure and biological effect. This fact gave an impetus to a number of seminal chemical ideas in the field of organic synthesis and favoured the development and perfection of physicochemical methods for the analysis of organic compounds that are equally used in organic and biological chemistry. Indeed, many marine toxins became the target compounds of multi-step syntheses that resulted in novel strategies, reagents and stereospecific reactions. In structural studies of these substances, innovative NMR spectroscopy and mass spectrometry techniques were assayed for the first time, new approaches to the determination of absolute configurations of chiral centres in organic compounds were developed that combine the synthesis of model compounds and physicochemical analysis. Besides, these studies led to the development of several drugs and affected the modern concepts of natural chemical defence and attack agents used in living systems.

Investigation into biological effects of toxins and molecular mechanisms of their action played an essential role in studying the molecular mechanisms of nerve impulse transmission and functioning the transport systems in biomembranes. This research exerted a profound influence on the development of such biological sciences as physiology and medicine, biochemistry and chemical ecology.

At the same time, there are also unsolved problems associated with the study of marine toxins. For example, not quite obvious is the biogenesis of some of them, in particular, the issue of the true producer of a number of highly potent polyether toxins. Are they indeed biosynthesized by microalgae or still by symbiont marine bacteria? The bacterial origin is also suggested by the similarity of such substances with polyether bacterial antibiotics and the presence of toxic and non-toxic lines of microalgal producers of these substances. Recently, when studying the genome of non-axenic strain of the dinoflagellate *Karenia brevis*, three genes encoding polyketide synthases, *i.e*., enzymes, which catalyze the biosynthesis of brevetoxins, were found and amplified. Their similarity to the genes discovered in one of the representatives of the protozoan *Cryptosporidium parvum* was demonstrated. The localization of these genes was determined by polymerase chain reaction (PCR), flow cytometry and fluorescent hybridization *in situ*. It was established that two out of these three genes are located exclusively in the microalgal cells, however the third was found in both these cells and the bacteria associated with this microalga.[143]

Still topical is also the question of fast and reliable identification of toxic microalgal species in phytoplankton samples. Fluorescence hybridization *in situ* and DNA-chip technology were proposed to resolve this problem,[144, 145] however, they did not find wide application so far.

In most cases, it is unclear what molecular defence mechanisms are used by producers and macroorganisms accumulating toxins to protect themselves from the action of these toxins. The majority of marine animals are sensitive to marine toxins. For example, in the late 1980s numerous instances of whale deaths brought on by saxitoxin poisoning were registered at the north-east coast of the US.[146] The blood of marine mammals is predominantly supplied to brain and heart, as a result, these organs are in the first place susceptible to the action of toxins. Saxitoxin and its derivatives are infrequently attributed to the instances of mass fish death. Thus mass herring death in summer 1976 in the Bay of Fundy was caused by PSP-toxins.[146] The herring death happened before the content of saxitoxin in its tissues reached a level dangerous for humans. At the same time,

molluscs and other invertebrates accumulating marine toxins in their tissues normally do not suffer from their action.

The production of marine toxins by marine organisms is governed, apparently, by many factors. In the case of microalgae, they include deficiency in some nutrient elements (especially, silicon and phosphorus), physical (temperature, illuminance) and biological (the influence of bacteria) effects.[147] Thus the ability of diatoms to synthesize domoic acid, as a rule, is in inverse proportion to their primary metabolic activity. Experimental evidence confirms the point of view according to which the production of domoic acid is a reaction to physiological stress experienced by diatoms.[147] The stress obviously results in an induction of the expression of genes encoding enzymes that synthesize domoic acid. However, molecular mechanisms that mediate the impact of a variety of factors on the production of this and other toxins are unknown so far.

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