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Reviews

Marine bioorganic chemistry as the base of marine biotechnology*

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Studies carried out at the Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences and at other centers of structural investigation of marine organism metabolites were used as examples to consider some features of the biochemistry of marine natural products and the achievements of marine bioorganic chemistry, which open up ways to the development of new drugs and biological preparations.

Key words: marine natural products, structure, physiological activity, bioorganic chemistry, biotechnology, biological preparations.

Introduction

Seas and oceans cover 71% of Earth´s surface and represent an enormous reservoir of life. According to prognistic estimates, about 20 million species of various organisms live in the ocean, although only 160 thousands of them have been reliably described to date by marine biologists. Nor even an approximate number of species of marine microorganisms, especially bacteria, is currently known, because for most of them, cultivation conditions have not been selected. Almost all the main large taxa have been found in seas and oceans, for example, 32 of 34 phyla of animals are represented here, 14 of these being purely marine inhabitants (foraminifera, echinoderms, bryozoans, and other). Representatives of ~90% of all known classes of animals and plants live in seas and oceans. Despite the fact that marine biota* is substantially inferior to terrestrial biota in the number of species (0.8—1.4 million species of insects and 270 thousand species of terrestrial green plants have been described),** it is considered that ~70—80% of Earth´s inhabitants live in sea water. The biological diversity of marine biota implies also chemical diversity of the corresponding primary and secondary metabolites and, naturally, attracts attention of not only biologists but also chemists.

Up to the early 1970s, the chemistry of natural products has been represented almost exclusively by the chemistry of terrestrial higher plants and soil microorganisms.

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* Biota is the animal or plant life of a region.

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The invention of an aqualung and the development of the scuba diving equipment, which made thousands of marine species living down to depths of 30—40 m available for collection, changed the situation and resulted in pioneering studies on pigments, sterols, and nucleosides of marine organisms. The sharp increase in the number of chemical studies of marine natural products observed from the late 1960s to 1980s was to some extent related to the development and further perfection of advanced laboratory isolation and analytical techniques, for example, high-performance liquid chromatography including that on so-called reversed-phase sorbents, various versions of mass spectrometry, 2D NMR, *etc*. An important role was played by construction of specialized research vessels and by sea expeditions aimed at collecting biological material for structural investigations. Many of these, including trips of the Soviet research vessel "Academician Oparin", were international. The foundation of new marine stations in some countries (USA, Italy, Japan, USSR) and involvement of existing stations in the research of marine natural products proved to be exceptionally useful for the development of this field, which is not restricted to the search, structural investigation, and synthesis of new marine natural products but devotes more and more attention to biological functions of the isolated substances. Currently, this scientific field is commonly referred to as marine bioorganic chemistry.

Marine bioorganic chemistry is tightly connected with marine biotechnology, whose rapid progress is regularly discussed at international conferences on marine biotechnology. Marine biotechnology is based on the practical use of biochemical transformations occurring in marine organisms, whilst the compounds resulting from these transformations represent the subject of investigation of marine bioorganic chemistry.

Biotechnology is known to be a science dealing with the use of biochemical processes in engineering and industrial production, mainly to produce drugs, food additives, agricultural preparations, vaccines, pigments, research chemicals, diagnosticums, and so on. In the broader sense of this term, production of any useful substances from biological (including microbial) raw materials and by enzymatic transformations are classified as biotechnological processes. In addition, the scope of biotechnology includes the creation of recombinant strains of microorganisms, cell and tissue cultures, transgene plants and animals, and so on.

This paper gives a survey of some features and achievements of marine bioorganic chemistry and an analysis of potential biotechnological applications of these achievements. The examples cited are mainly results of investigations on marine organisms carried out at the Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences (PIBOC).

General results of structural research into marine natural products. Their structural singularity and diversity

At present, ~14 to 15 thousand marine natural products (low-molecular-weight bioregulators) are known. Almost all of them are new and have not been found previously in terrestrial objects. What are the reasons for this singularity of secondary metabolism processes in marine organisms? The main reason is, evidently, the taxonomic difference between the terrestrial and marine biotas. Indeed, the major producers of natural products on land are higher plants, while in the sea and ocean water mass, these are marine microalgae and bacteria, and on the ocean bottom, the marine invertebrates and lower plants (algae). Thus, the main taxa of the organisms predominating on land and in the sea are essentially different, and the routes of their historical development parted hundreds of millions years ago.

In addition, the biochemical reactions leading to so-called natural products in marine organisms have been substantially affected by peculiar conditions of their existence. Marine organisms live in the sea environment rather than in air, often absolutely or almost without sunlight, under pressures reaching thousand of atmospheres, and at a relatively narrow temperature gradient.

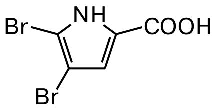
It is of interest that during biosynthesis, marine natural products often incorporate components of the marine environment such as halide, sulfate, and cyanide ions, *etc*. Brominated secondary metabolites are especially frequently encountered; these include simple molecules such as bromoform, dibromomethane, and so on, synthesized in enormous amounts by some aquatic species.[1] In addition, the enzymes that perform halogenation by the reaction

$$\mathrm{OrgH} + \mathrm{H_2O_2} + \mathrm{X^-} + \mathrm{H^+} \longrightarrow \mathrm{X{—}Org} + 2\,\mathrm{H_2O},$$

i.e., marine haloperoxidases,[2] are able to halogenate substrates differing in chemical nature, giving rise to substantial diversity of halogenated marine natural products.

Marine halogenated metabolites can be exemplified by alkaloids, namely, 4,5-dibromopyrrole-2-carboxylic acid (**1**) from sponges of the Axinellidae and Agelasidae families,[3] dibromoisofakelin (**2**) from the sponge *Acanthella carteri*,[4] dibromoagelospongine (**3**) from the sponge *Agelas* sp.,[5] pibocins A (**4**) and B (**5**) from the ascidian *Eudistoma* sp.,[6,7] derivatives of diphenyl ethers **6—11** from a number of *Dysidea* sponges,[8,9] new dibenzodioxins **12—15** from the sponge *Dysidea dendyi*,[10,11] and sesquiterpenoids **16**, **17** from the marine shellfish *Aplysia dactylomela*.[12,13]

Sulfur (mainly, as sulfate) is the fourth most abundant element in sea water after chlorine, sodium, and magne-



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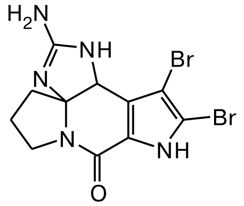

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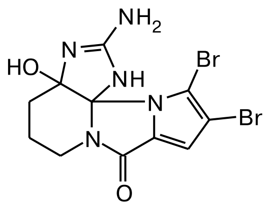

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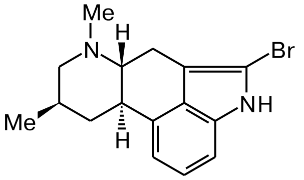

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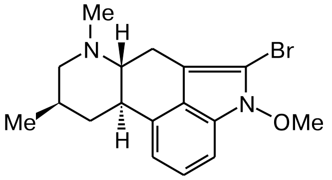

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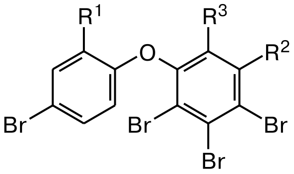

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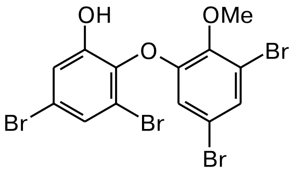

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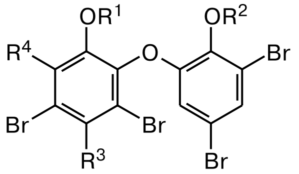

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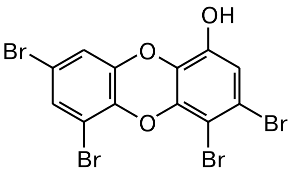

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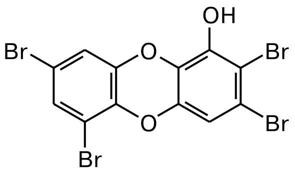

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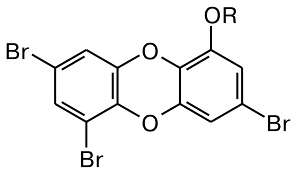
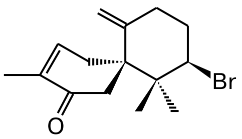


14: R = H
15: R = Me

	6	**7**	**9**	**10**	**11**
R¹	Br	Br	Me	H	H
R²	H	Br	Me	H	H
R³	OH	OH	H	H	Br
R⁴			H	Br	Br



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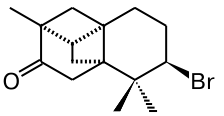

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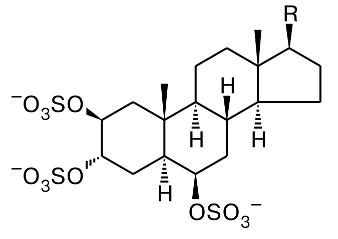
sium. Sulfation of quite diverse substrates ranging from polysaccharides to terpenoids is a typical feature of the biochemistry of secondary metabolism in marine organisms. Biochemical systems that sulfate various substrates function in marine microorganisms, algae, many types of marine invertebrates, hemichordates, and fishes. More than five hundred low-molecular weight marine natural products bearing one to three sulfate groups have now been described.[14,15] In addition, a large number of marine secondary metabolites contain a polysulfide fragment.

Sulfur-containing marine metabolites have been repeatedly isolated by researchers from the PIBOC. For example, a series of trisulfated steroids from sponges was found. The most unusual of these is sokotrasterol sulfate (**18**) from a nonidentified species of the Halichondriidae family,[16] which differs from other steroids not only in extensive sulfation but also in the side-chain structure. The side chain contains three methyl groups more than sterols of the ergostane series, which are close structural



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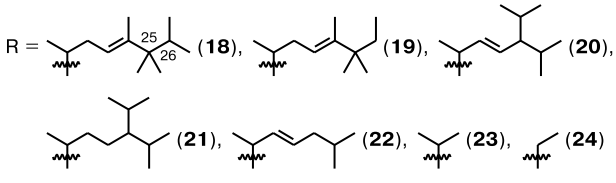

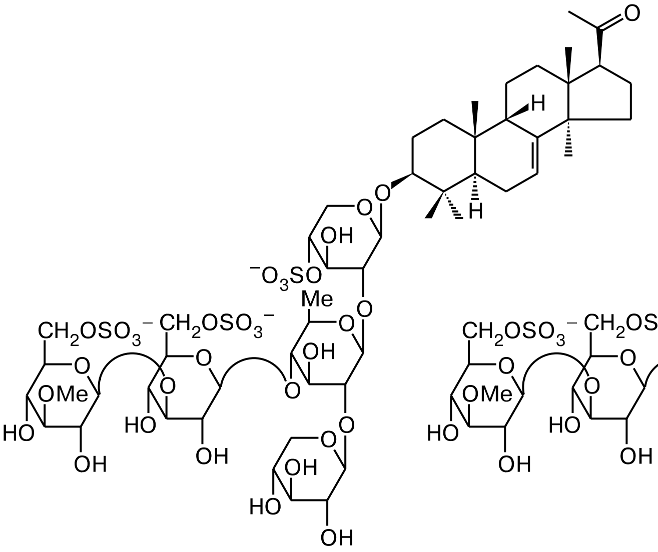

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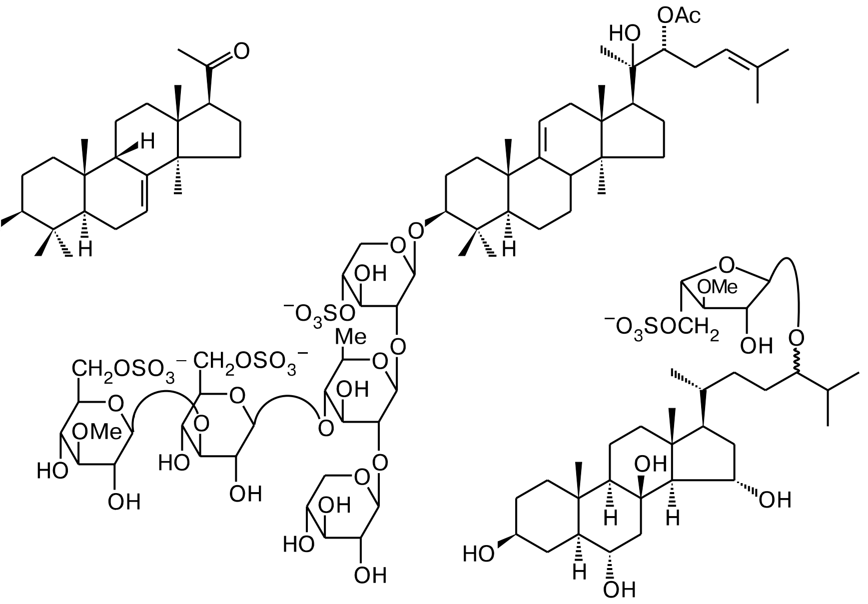

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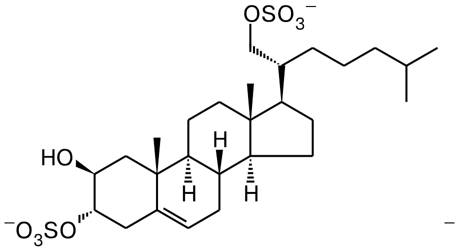

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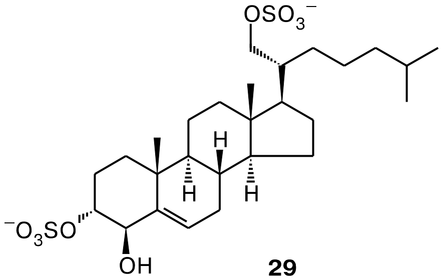

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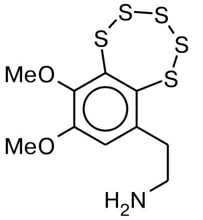

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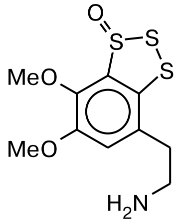

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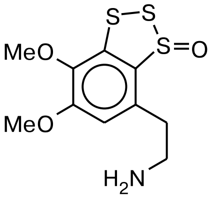

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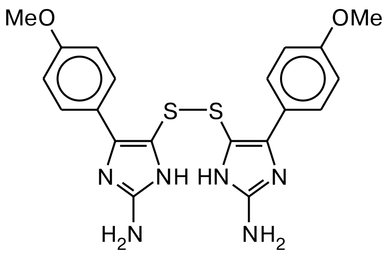

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relatives of this trisulfate. These methyl groups are attached to the C(25) and C(26) atoms; this is highly improbable for steroids of the terrestrial origin. Later, compound **18** has been found time and again by foreign researchers in various species of sponges. Other compounds having similar structures are norsokotrasterol (**19**),[17] 24-isopropylcholest-22-ene-2β,3α,6β-triol trisulfate (**20**) from *Trachiopsis halichondroides*,[18] and steroids **21**—**24** from the *T. halichondroides* and sponges *Cymbastella coralliophila*.[19]

Sulfated metabolites are widely represented in various echinoderms; in holothurians, these are mainly triterpene glycosides such as koreoside A (**25**) from *Cucumaria koraiensis*[20] or frondoside C (**26**) from *Cucumaria frondosa*.[21] We determined the structures of more than eighty new glycosides from holothurians (see reviews[22—26]). Asterosaponin P_1 (**27**) from the starfish *Asterina (Patiria) pectinifera*[27] and steroids **28**, **29** from the ophiuroid (brittle star) *Ophiura sarsi*[28,29] are examples of sulfated derivatives.

Polysulfides are encountered more frequently in ascidia and in red algae. For example, varacin (**30**) and its derivatives **31**, **32** were found in the Far-Eastern ascidian *Polycitor* sp.[30] Varacin is a cytotoxic benzopentathiepine

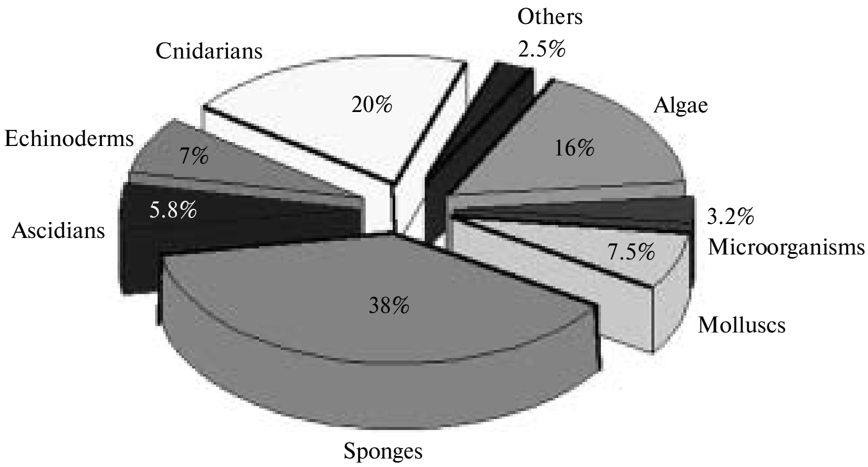


Fig. 1. Major producers of known marine natural products (% of the total number of compounds).

possessing antibacterial and antitumor properties, and derivatives **31** and **32** are oxidized benzotrithioles.

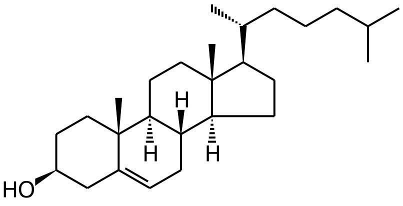
The antitumor disulfide-type alkaloid polycarpin (**33**) was found in the ascidian *Polycarpa aurata* collected from the Great Barrier Reef and studied by X-ray diffraction analysis; later, this compound was synthesized together with a series of its structural analogs.[31,32]

The singularity of the marine secondary metabolites is combined with their structural diversity. Representatives of all the main classes of natural products have been found in marine invertebrates and algae: terpenoids and steroids, alkaloids and peptides, various aliphatic compounds including oxylipins, phenol and quinone derivatives, various pigments, alkaloids, polyethers, and many other compounds. Increasing numbers of unusual secondary metabolites have been isolated in recent years from marine microorganisms: bacteria,[33] fungi, and microalgae.[34] *

The greatest number of secondary metabolites was isolated from the most primitive multicellular animals, namely, sponges (Fig. 1).

Unlike protozoa colonies, they contain differentiated cells and tend to form tissues. However, sponges have no nervous system and no real organs. Sponges are the only animals that belong to the Parazoa subkingdom; during individual development, the primordial leaves (outer and inner) interchange. All other animals form the Eumetazoa subkingdom. In addition, sponges are typical symbiotic systems; their cells and intercellular medium are normally populated by microbial symbionts such as Cyanophyceae (cyanobacteria), archaebacteria, *etc*. In some species, the volume occupied by these unicellular organisms is comparable with the cell volume of the host itself (*i.e.*, sponge).

The diversity of secondary compounds in sponges can be conceived, for example, by comparing their sterol components with those in higher animals. Sterols, or steroid alcohols are components of biomembranes and biosynthetic precursors of all other steroids. The predominant sterol in animals and other mammals is cholesterol (**34**). The other sterols usually account for fractions of a percent.



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Unlike terrestrial animals, sponges were found to contain a multitude of unusual sterols, the sterol fractions consisting of numerous components; in some cases, these can contain 50—70 compounds, most often, with different side chains.[36—38] Compounds **35—41**, isolated from different sponges: *Halichondria* sp.,[39] *Halichondria* sp.,[40,41] and *Rhizochalina incrustata*[42] are given here as examples of new unusual sterols.

Some other marine invertebrates, in particular, echinoderms, are also well known for their complex sterol composition.[43—48] New core-methylated sterols **42** and **43**, which are the major sterol components of some holothurian species, were found by researchers from the PIBOC.[49,50]

The substantial chemical diversity of marine biomolecules is, apparently, due to some extent to the antiquity of their producers (long period of the evolutionary search for chemical means of adaptation to the environment conditions). Biologists are familiar with the fact that the greatest diversity of morphological features is peculiar to representatives of ancient taxa that underlie the phylo-

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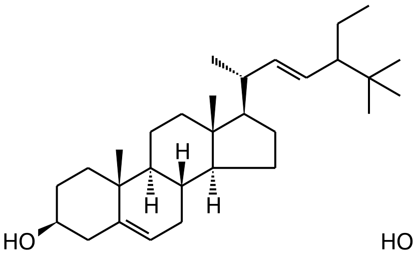
* Serial reviews on marine natural products were published annually by Prof. D. J. Faulkner (Scripps Institute of Oceanography, University of California at San Diego, USA).[35]



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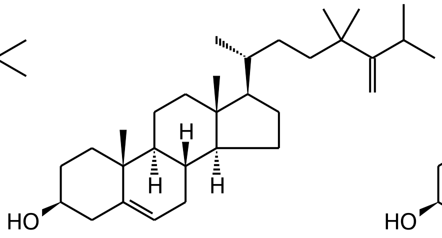

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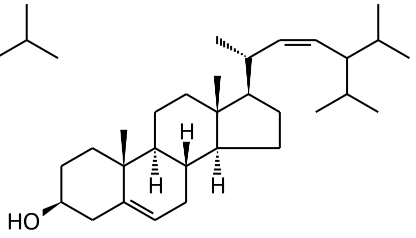

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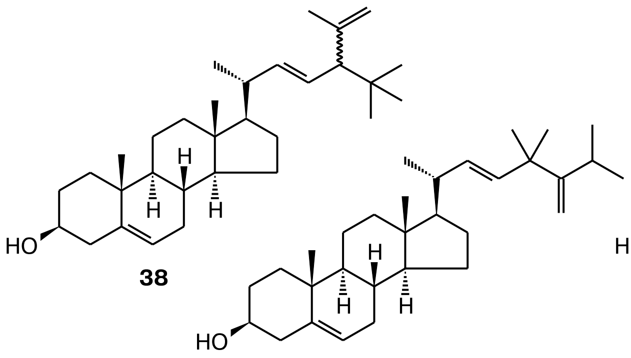

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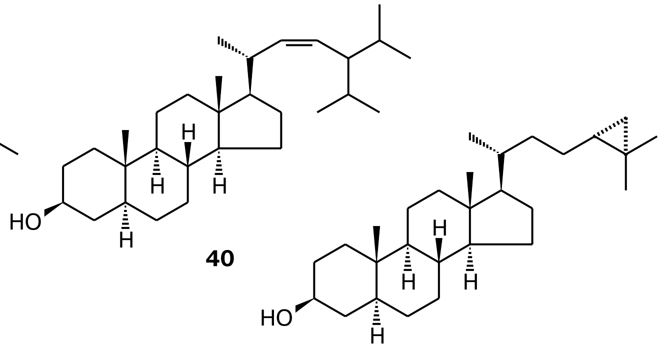

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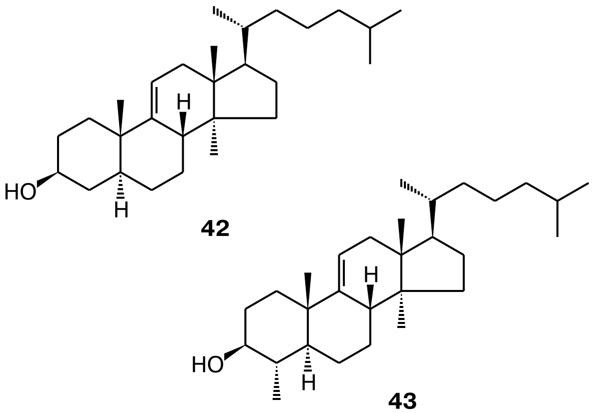

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genetic tree. Apparently, this rule is also applicable to the biochemical diversity. Indeed, Coelenterata, sponges, and echinoderms appeared on the Earth more than 400 million years ago; they are substantially more ancient than terrestrial animals or plants. The secondary metabolites of these animals are much more chemically diverse than those in mammals (see Fig. 1).

However, the secondary compounds in terrestrial plants are more diverse than those in lower plants (algae); this might be due to the much fewer number of species of the latter and to the fact that the chemical composition of the vast majority of these species, especially microalgae, has been poorly studied.

It is of interest that highly polar and water-soluble components are more abundant among the marine natural products than among secondary metabolites of the land origin. The so-called exometabolites secreted to the medium (which comprise protective, signaling, and other substances including pheromones and allomones*) play a rather important role for marine animals, because other communication means (vision, audible signals) are less applicable for sea bosom inhabitants than for terrestrial organisms due to the deficiency of light and high density of the medium. Marine exometabolites have to be water-soluble to diffuse in the sea water from the donor to a recipient of the chemical signal.

Therefore, polar glycosides, alkaloids, and various polyhydroxylated derivatives (some of them performing protective functions) are often found in marine organisms. The chemical structures of the marine metabolites belonging to these chemical groups differ substantially from their analogs isolated from terrestrial higher plants.

Thus the marine glycosides found in the echinoderms and sponges differ from those isolated from terrestrial animals in both the aglycon and carbohydrate components. Glycosides from holothurians are mainly derivatives of a hypothetical triterpene alcohol holostanol (**44**) with a characteristic (18→20) lactone fragment. Their carbohydrate chains, often sulfated, incorporate rare monosaccharides such as 3-*O*-methylglucose, 3-*O*-methylxylose, and quinovose. For example, cucumariosides from the Far-Eastern commercial holothurian *Cucumaria japonica*[51–54] with the general formula **45** are holostanol derivatives in which the fusion pattern of the rings *B* and *C* is unusual for steroids and triterpenes (9β-configuration)

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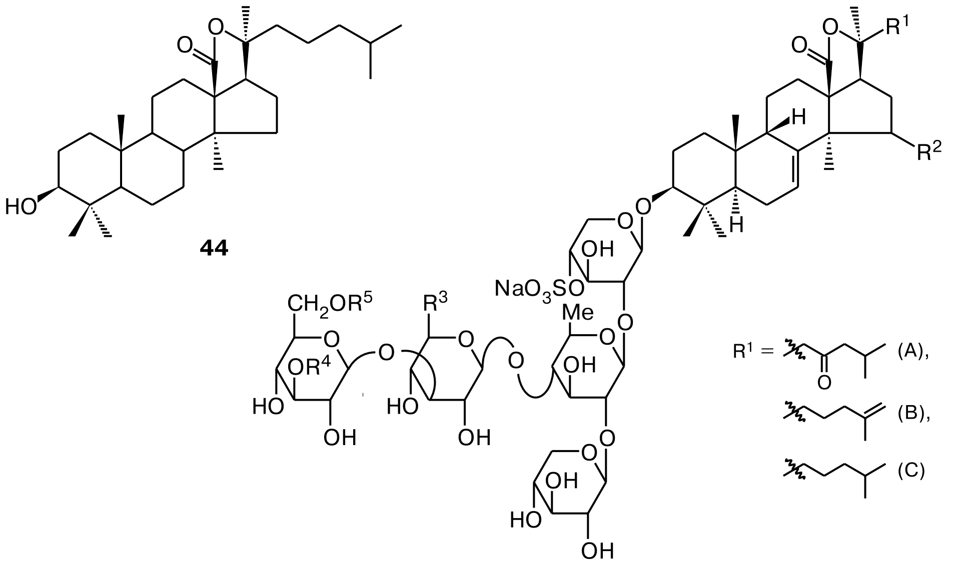
* Allomones are signal compounds exerting directed action on representatives of other species.



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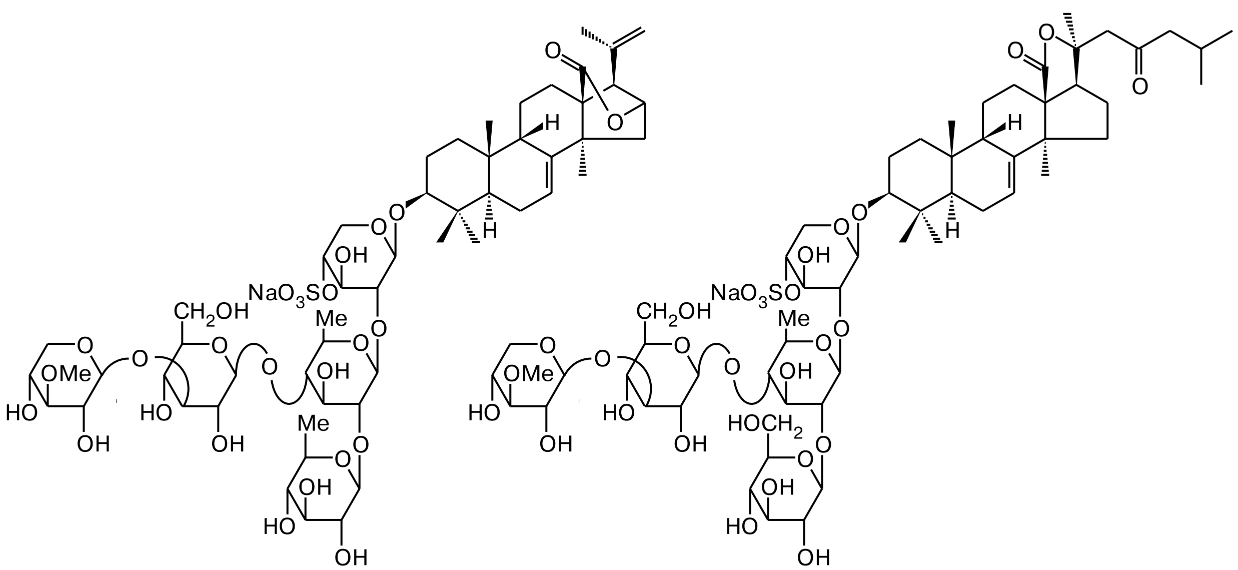
45a—m

	R^1	R^2	R^3	R^4	R^5
a	A	OAc	H	Me	H
b	B	OAc	H	Me	H
c	B	O	H	Me	H
d	B	O	CH_2OH	H	Ac
e	B	O	CH_2OH	Me	H
f	C	O	CH_2OH	Me	H
g	B	H	CH_2OH	Me	H
h	B	H	CH_2OH	H	H
i	B	O	CH_2OSO_3Na	Me	SO_3Na
j	C	O	CH_2OSO_3Na	Me	SO_3Na
k	B	H	CH_2OSO_3Na	Me	SO_3Na
l	B	O	CH_2OSO_3Na	Me	H
m	B	O	CH_2OH	Me	SO_3Na



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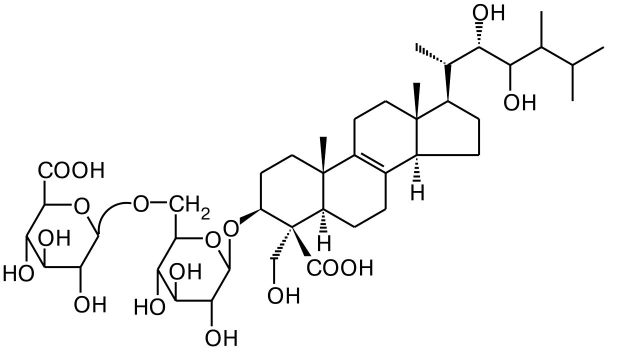
and whose pentasaccharide carbohydrate fragments bear xylose, glucose, quinovose, and 3-*O*-methylglucose residues. These compounds differ from one another in the number of sulfate groups, the structure of side chains, and substituents in the ring *D* of the aglycon. Recently, structures of holostane and nonholostane glycosides related to compound **45** with 3-*O*-methylxylose in the carbohydrate chain were reported; for example, glycosides **46** and **47** from the Far Eastern holothurian *Pentamera calcigera*.[55,56]

Glycosides from sponges are norlanostane or steroid derivatives with galactose, uronic acids, aminosugars, and D-arabinopyranose in the carbohydrate chains. They include ulososides,[57—59] for example, ulososides A and B (**48**, **49**) from *Ulosa* sp., mycalosides,[60] for example,



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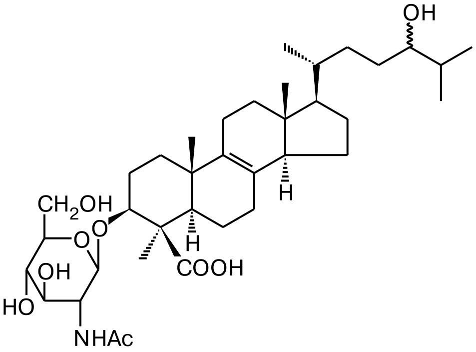

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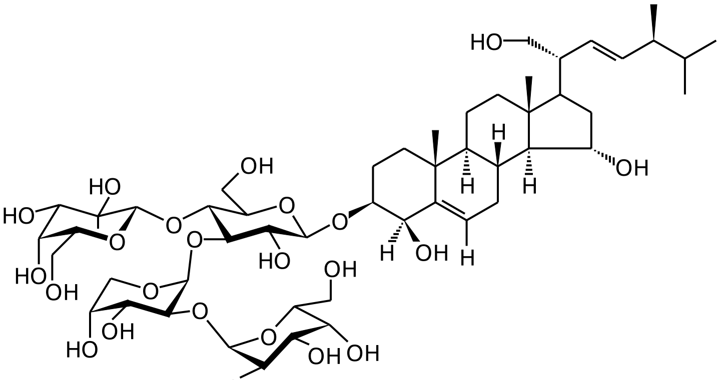


50

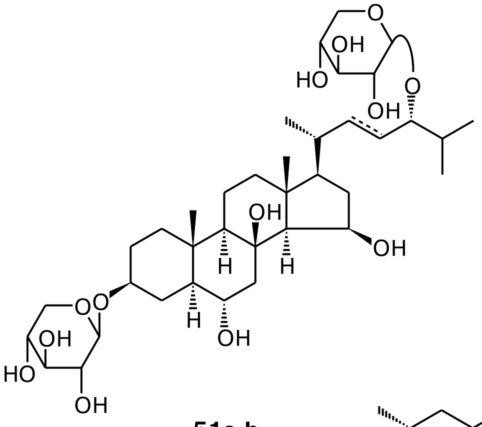
mycaloside A (**50**) from *Mycale laxissima*, and a number of other compounds identified at various laboratories in the world.[15] These compounds usually carry several polar groups (carboxy and/or hydroxy) in the aglycon.

Starfishes represent an exceptionally rich source of various steroid glycosides; their aglycons or the carbohydrate components are often sulfated; the latter contain quinovose, fucose, glucose, galactose, or xylose. Apart from oligoglycosides, mono- and biosides whose monosaccharides are often partially methylated are widely represented.[15,24,35,61] Distolasteriosides (**51a,b**) from *Distolasterias nipon*[62] and aphelasterioside C (**52**) from *Aphelasterias japonica*[63] are examples of such glycosides.

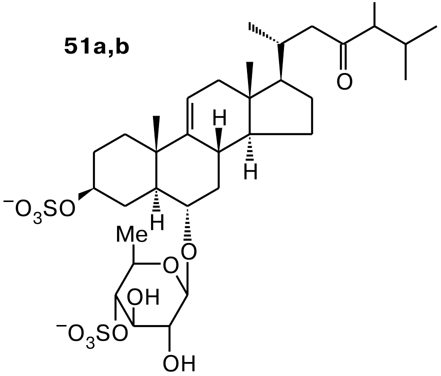
Altogether, researchers from the PIBOC have determined the structures of ~100 new glycosides and related compounds from starfishes.[15,24]

X-Ray diffraction analysis shows that compounds like distolasteriosides and, apparently, many other marine glycosides and polar marine metabolites have polar substituents arranged in such a way as to form a spatially well-confined hydrophilic area in space. The nonpolar part of the molecule, which is also quite clear-cut in space, is a hydrophobic area of the molecule. Thus, most of these polar metabolites are amphiphilic and many of them might function as signal compounds, as they react through their hydrophobic fragments with the corresponding receptors of recipients.

The marine environment contains little oxygen as compared with the air environment; nevertheless, many polar marine metabolites are literally saturated by oxygen atoms. Of these compounds, the greatest attention is



51a,b



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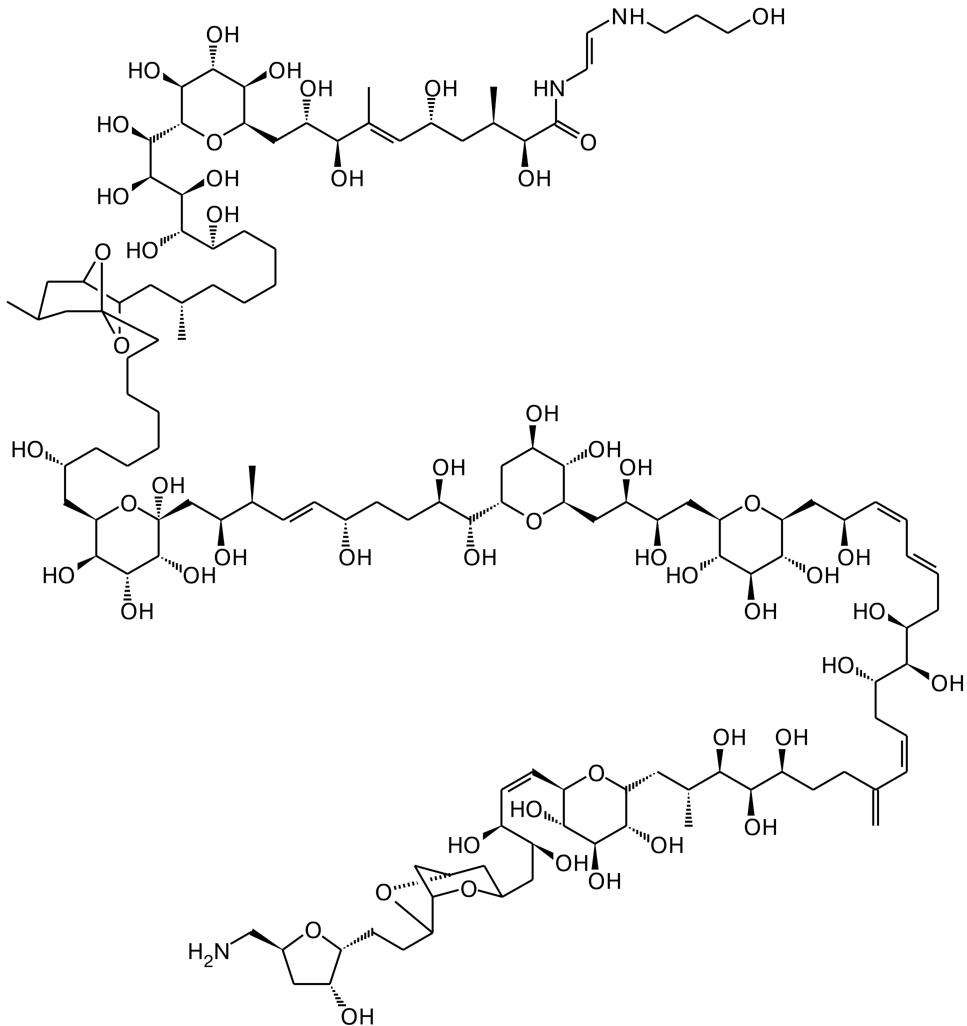
22(23)-dehydro (**a**), 22(23)-dihydro (**b**)



53

attracted by polyether derivatives including palytoxin (**53**), one of the most potent nonprotein toxins, first isolated by American scientists from hexacorals (zoantharians) *Palythoa*.[64]

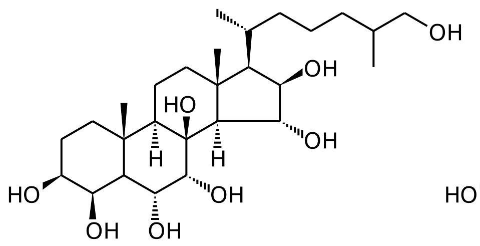
The determination of the structure of this exceptionally complicated compound[65,66] and, subsequently, its synthesis[67,68] have become outstanding events in the bioorganic chemistry of the XX century. Presumably, this and related toxins are produced by symbiotic microorganisms, *viz.*, microalgae. It is not surprising that these have repeatedly been found not only in Zoantharia but also in other marine animals: crabs,[69] sea anemones,[70] and fishes.[71] An immunoenzyme assay of palytoxin in various biological objects has been developed at our Institute.[72]

Many other secondary metabolites are also rich in oxygen atoms. We would like to mention octahydroxysterol (**54**) and nonahydroxysterol sulfate **55** from starfishes *Patiria* (=*Asterina*) *pectinifera*[73] and *Archaster typicus*,[74] respectively, highly oxidized terpenoids from Octocorallia, for example, sarcophytins B and C (**56**, **57**) from *Sarcophyton* sp.[75] and plumarellide (**58**) from *Plumarella* sp.[76], pigments from sea urchins and other echinoderms including spinochromes, for example **59**, echinochrome (**60**),[77] dimeric pigments **61** and **62**,[78,79] and an acetyl-containing pigment **63**.[80]

These examples indicate the presence, in marine organisms, of mono- and dioxygenases with exceptionally diverse substrate specificities. Indeed, the diversity of oxidation routes of steroid substrates found here is as broad as that in soil microorganisms. This is illustrated in the scheme below where arrows show the sites of possible oxidation for various steroid skeletal systems (**64**—**66**) in starfishes.

It is well-known that microbial oxidation of steroid derivatives is used to produce steroid drugs, whereas oxidation by enzyme, cell, or tissue preparations from marine organisms has not yet found its biotechnological application.

Recently, it was shown[81] that gorgosterol (**67**) was efficiently transformed into secosteroid (**68**), possessing cytostatic and antiinflammatory activities, by a total en-



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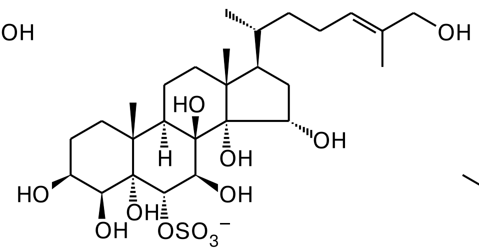

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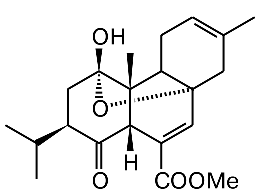

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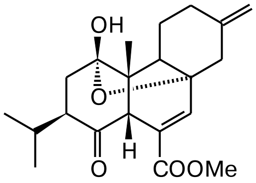

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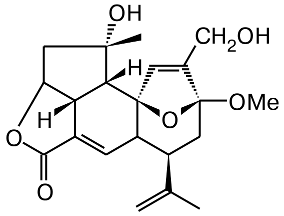

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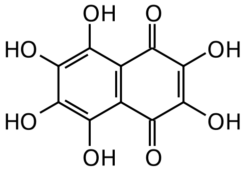

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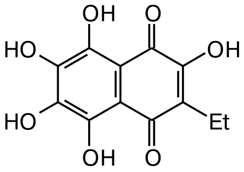

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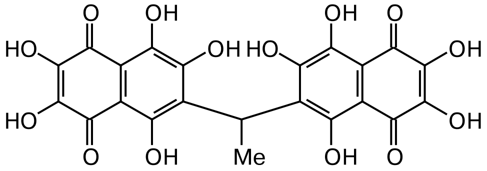

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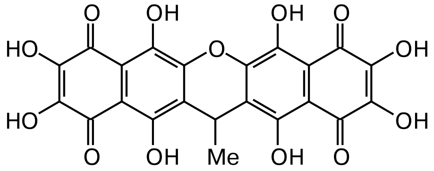

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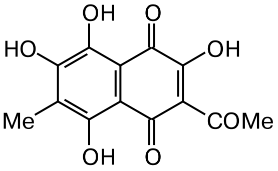

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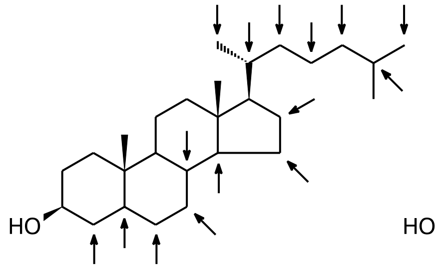

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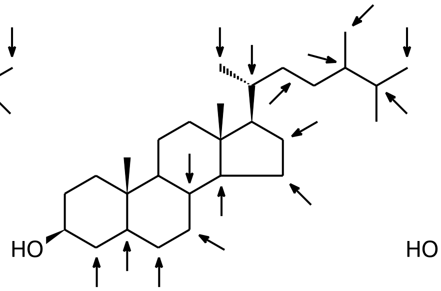

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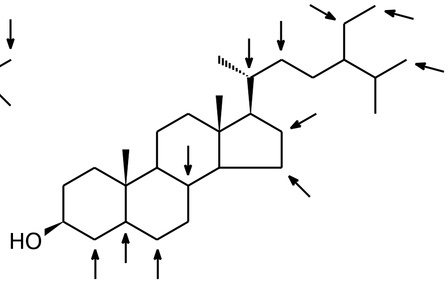
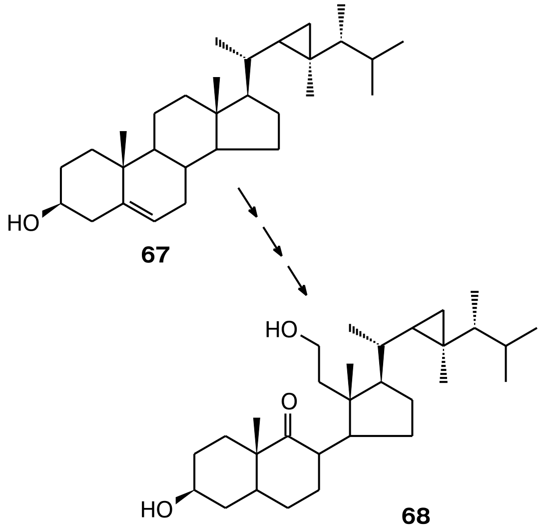


66

zyme preparation from the gorgonian coral *Pseudopterogorgia* sp. in the presence of NAD, NADP, and glutamate dehydrogenase. This and some other studies carried out at our Institute and devoted to transformations of sterols by starfish homogenates[82] confirm good prospects of using marine biosystems in biotechnology for oxidative transformations of readily accessible compounds into more valuable products.

Extensive oxidative processes that take place in marine invertebrates and microalgae might be due to the fact that active forms of oxygen are more toxic for them than for terrestrial animals or plants and are rapidly consumed for the oxidation of various low-molecular-weight substrates, whose oxidized derivatives thus remove excess oxygen from the organism.

The chemical diversity in the marine biota is characterized by abundance of the so-called mixed biogenesis



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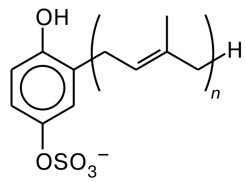
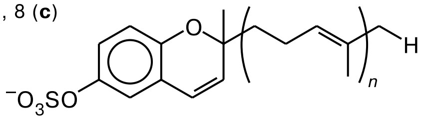


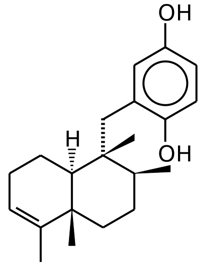
69a—c

n = 6 (**a**), 7 (**b**), 8 (**c**)



70a—c

n = 5 (**a**), 6 (**b**), 7 (**c**)



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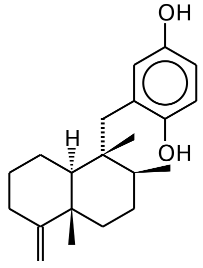


72

products. In addition, numerous previously unknown groups of compounds with new types of skeletal systems have been found in marine organisms. Among the products of mixed biogenesis in marine organisms, terpenoid derivatives with phenolic, quinoid, nitrogen-containing, or aliphatic fragments are especially abundant. As an example, one can consider the structures of so-called sarcohydroquinone sulfates (**69a—c**) and sarcochromenol sulfates (**70a—c**) from the sponge *Sarcotragus spinulosus*,[83] which are Na,$^+$K$^+$-ATPase inhibitors, or the structures of avarol (**71**)[84] and isomeric isoavarol (**72**),[85,86] which exhibit toxicity for tumor cells and an anti-HIV activity.

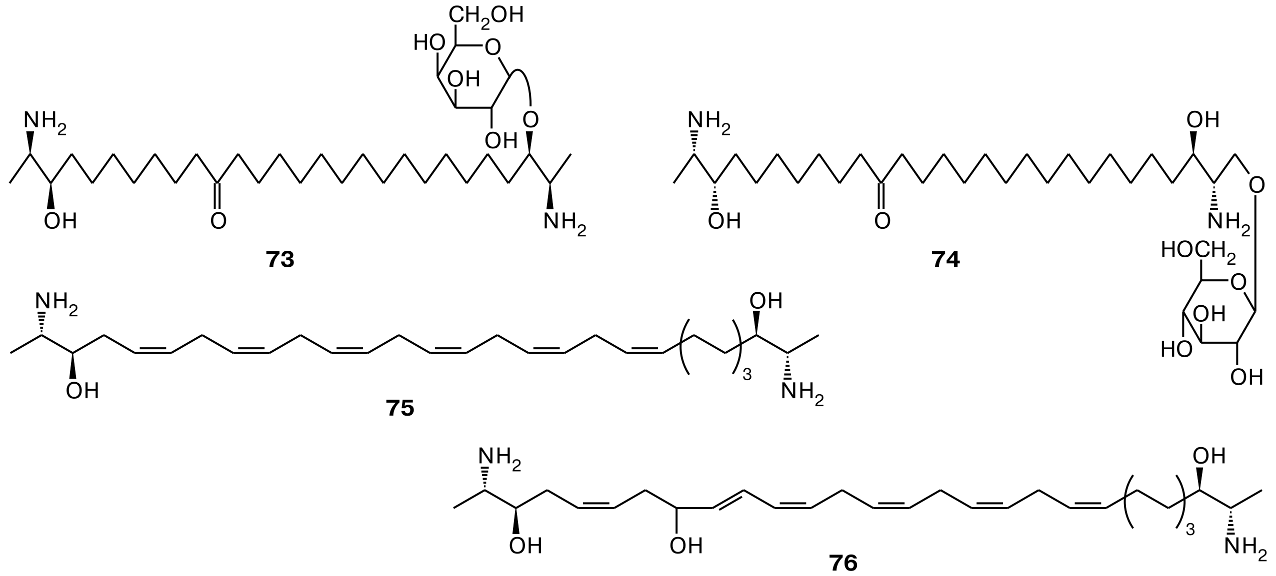
Rhizochalin (**73**) isolated from sponge *Rhizochalina incrustata*[87] proved to be the first representative of new so-called "two-headed" (bipolar) sphingolipids. The absolute configuration of this compound has been recently established by circular dichroism.[88] Now the number of such compounds has increased and includes several glycosylated and nonglycosylated compounds with sphingo-like ends of molecules containing serine and/or alanine residues, for example, calycoside (**74**) from *Calyx* sp.[89] and leucettomols (**75**, **76**) from *Leucetta microraphis.*[90]

Biopolymers from marine organisms

Marine biopolymers including primary metabolites have been studied much less extensively than low-molecular-weight bioregulators. However, in this case, too, substantial taxonomic differences between the terrestrial and marine biotas should entail structural peculiarity and diversity of these biopolymers.

Research into enzymes from marine organisms is a promising route of development of marine bioorganic chemistry, closely related to biotechnology. The studies carried out at the PIBOC were focused on hydrolytic enzymes, which participate not only in cell metabolism but also in utilization, by marine organisms, of exogenous biopolymers as important sources of phosphorus, nitrogen, and carbon in marine ecosystems. Particular attention was devoted to enzymes that cleave polysaccharides from algae.

The enzymes *O*-glycoside hydrolases that cleave water-soluble polysaccharides, laminarans, (laminarinases) are widely distributed in marine organisms, but only the digestive glands of molluscs (the so-called crystalline style) proved to be unique accumulators of such substances.[91]



73

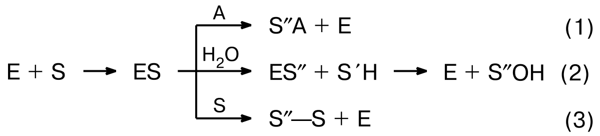
74

75

76

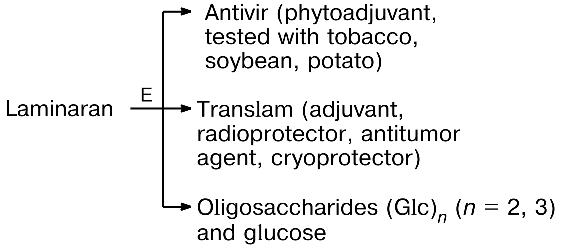
The laminarinases from the crystalline style of commercial molluscs *Spisula sachalinensis* (L_{IV}) and *Chlamys albidus* (L_o) have been comprehensively studied. For one of them (L_{IV}), the primary structure was determined by the molecular cloning method and for both of them, the molecular weights were determined and the catalytic properties were studied including the spatial and energetic characteristics of the active sites.[91—94] In terms of the type of action on the substrate, many enzymes of this group belong to endoglycanases, which cleave hydrolytically the internal glycosidic bonds in polysaccharides. A distinctive feature of these enzymes, as compared to glycanases from terrestrial sources, is the enhanced transglycosylation activity (Scheme 1, reaction (1)), *i.e.*, the transfer of residues formed upon hydrolysis of oligosides to an appropriate acceptor (in Scheme 1, it is designated by A). In addition, they were found to exhibit glycanosyl transferase activity, the resulting polysaccharides having a higher molecular weight and being more branched than the initial substrate (S) (see Scheme 1, reaction (3)). Thus, these enzymes catalyze three types of reaction, namely, transglycosylation (1), hydrolysis (2), and glycanosyl transferase reaction (3).

Scheme 1



Using the glycanosyl transferase properties of laminarinases, several new polysaccharides were prepared from laminaran by enzymatic synthesis, for example, "Antivir," which has shown phytoadjuvant properties in field trials with various crops, and "Translam", which has been tested as a radioprotector for several years (Scheme 2).[95]

Scheme 2



Of enzymes that depolymerize nucleic acids, Ca^{2+},Mg^{2+}-dependent and acidic metal-independent DNases are frequently encountered in marine invertebrates.[96,97] The former were prepared from embryos of the sea urchin *Strongylocentrotus intermedius* and from the king crab *Paralithodes camtchatica*.[97,98] These enzymes differ from the previously known metal-dependent DNases, in particular, from the pancreatic DNase, in the structure and properties. Thus they have a 1.5 times higher molecular mass and an isoelectric point at pH 4.0, and the DNase from *P. camtchatica* had a high thermal stability and withstood a 10-min heating at 100 °C, which was, obviously, due to the large number of disulfide bridges, namely 18, in the molecule.[96,98] Marine DNases possess clearly pronounced specificity with respect to the secondary structure of substrates: the enzymes mainly hydrolyze double-stranded DNA, which makes them valuable biochemical tools. They can be employed as versatile restrictases which may recognize double-stranded fragments on a given site of single-stranded DNA using synthetic oligonucleotides.

Marine acidic DNases including those from walleye pollock milt and sea-urchin embryos[96] hydrolyze both double-stranded and single-stranded DNA, mainly in the Pur-Pyr and Pur-Pur sequences.

Marine bacteria proved to be a perfect source of a variety of enzymes including highly active alkaline phosphatases[99] and carbohydrases[100] as well as polyuridyl endoribonuclease,[101] α-*N*-acetylglucosaminidase,[102] α-galactosidases,[103] *etc.*

Investigations into peptide neurotoxins from various marine invertebrates that react with excitable membrane channels have played an important role in the study of the chemical fundamentals of nerve conduction. The isolation of five so-called "long" neurotoxins from the sea anemone *Radianthus macrodactylus* is an example of this type of study. They are representatives of a new type of anemonotoxins. The structures of these toxins[104—107] are stabilized by three intramolecular disulfide bonds. Like other anemonotoxins, they retard inactivation of fast sodium channels and transfer some of the channels into a non-inactivatable form.[108,109] The positively charged amino acid residues play an important role in binding of these substances to the channel, the binding itself being multiple-site.[110,111] One toxin molecule is required to modify one channel. In terms of the type of physiological action, they are analogs of α-scorpionotoxins.

Polysaccharides from algae are known to differ substantially in the structure and properties from compounds of the same class from terrestrial organisms. Many of them, in particular, carrageenans, and agars from red algae[112—114] and fucoidans from Phaeophyceae[115—117] contain sulfate groups.

Evidenty, a certain structural peculiarity is also inherent in antigenic polysaccharides of marine bacteria and, hence, in cell walls of these bacteria, which contain these polysaccharides. Thus it was shown that cell walls of a number of strains of marine bacteria studied either do not contain classical lipopolysaccharides or contain lipopolysaccharides with abnormally low contents of the lipid component. Polysaccharides of this type often incorporate rare and unusual acid monosaccharides and *N*-acylaminosugars.[118]

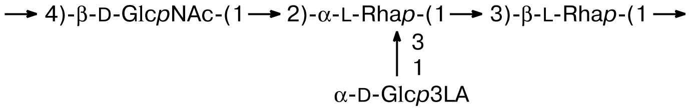
For example, a new branched monosaccharide, shewanellose, has been found in a phenol-soluble polysaccharide. Below we present the structure of a repeating unit (**77**) of a polysaccharide from *Shewanella putrefaciens* A6 [119]

$$\rightarrow 4)\text{-}\alpha\text{-Leg-}(2 \rightarrow 3)\text{-}\beta\text{-She-}(1 \rightarrow,$$

77

where Leg is 7-acetamidino-5-acetamido-8-*O*-acetyl-3,5,7,9-tetradeoxy-L-*glycero*-α-D-*galacto*-non-2-ulosonic acid, and She is 2-acetamido-2,6-dideoxy-4-C-(3-carboxamido-2,2-dihydroxypropyl)-β-D-galactopyranose (shewanellose).

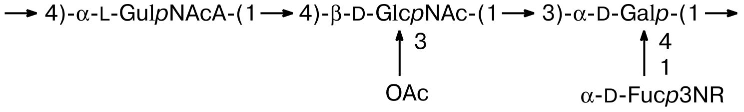
Polysaccharides from *Pseudoalteromonas haloplanktis* KMM 156 (with a repeating unit **78**),[120] *P. haloplanktis* KMM 158 (repeating unit **79**),[121] *P. nigrifaciens* IAM13010T (**80**),[122] *P. marinoglutinosa* KMM 232 (**81**),[123] *P. haloplanktis* KMM 223 (**82**),[124] *Shewanella putrefaciens* S29 (**83**),[125] and *S. alga* 48055 (**84**)[126] also have, most often, unusual chemical structures. For example, the polysaccharide **78** contains the residue of an unusual monosaccharide, *viz.*, 3-*O*-[(R)-1-carboxyethyl]-D-glucose, and the polysaccharide **79** contains two rare monosaccharides, *viz.*, 3-amino-3,6-dideoxy-D-galactose N-acylated by 4-hydroxybutyramidic acid and 2-amino-2-deoxy-L-guluronic acid.



$$\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1 \rightarrow 2)\text{-}\alpha\text{-L-}\underset{\substack{\uparrow 3\\ |\,1\\ \alpha\text{-D-Glc}p\text{3LA}}}{\text{Rha}p}\text{-}(1 \rightarrow 3)\text{-}\beta\text{-L-Rha}p\text{-}(1 \rightarrow$$

78

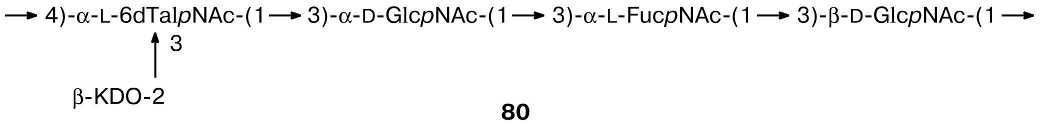
LA is lactic acid residue



$$\rightarrow 4)\text{-}\alpha\text{-L-Gul}p\text{NAcA-}(1 \rightarrow 4)\text{-}\beta\text{-D-}\underset{\substack{\uparrow 3\\ |\\ \text{OAc}}}{\text{Glc}p\text{NAc}}\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-}\underset{\substack{\uparrow 4\\ |\,1\\ \alpha\text{-D-Fuc}p\text{3NR}}}{\text{Gal}p}\text{-}(1 \rightarrow$$

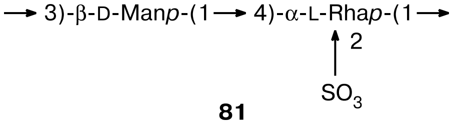
79

R = CO—$(CH_2)_3$—OH



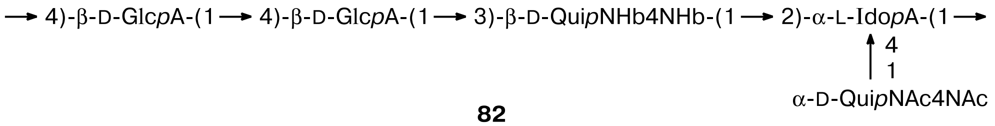
$$\rightarrow 4)\text{-}\alpha\text{-L-}\underset{\substack{\uparrow 3\\ |\\ \beta\text{-KDO-2}}}{\text{6dTal}p\text{NAc}}\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1 \rightarrow 3)\text{-}\alpha\text{-L-Fuc}p\text{NAc-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1 \rightarrow$$

80



$$\rightarrow 3)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-L-}\underset{\substack{\uparrow 2\\ |\\ SO_3}}{\text{Rha}p}\text{-}(1 \rightarrow$$

81



$$\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A-}(1 \rightarrow 3)\text{-}\beta\text{-D-Qui}p\text{NHb4NHb-}(1 \rightarrow 2)\text{-}\alpha\text{-L-}\underset{\substack{\uparrow 4\\ |\,1\\ \alpha\text{-D-Qui}p\text{NAc4NAc}}}{\text{Ido}p\text{A}}\text{-}(1 \rightarrow$$

82

QuiNAc4NAc — 2,4-diacetamido-2,4,6-trideoxyglucose,
QuiNHb4NHb — 2,4,6-trideoxy-2,4-di[(S)-3-hydroxybutyramido]glucose

$$\rightarrow 4)\text{-}\alpha\text{-L-Qui}p\text{NAc-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{NAc-}(1 \rightarrow 3)\text{-}\beta\text{-D-Qui}p\text{4NAc-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-1-P-(O} \rightarrow$$

83

$$\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{A6GroN-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{A6GroN-}(1 \rightarrow 4)\text{-}\beta\text{-Neu}p\text{5Ac-}(2 \rightarrow$$

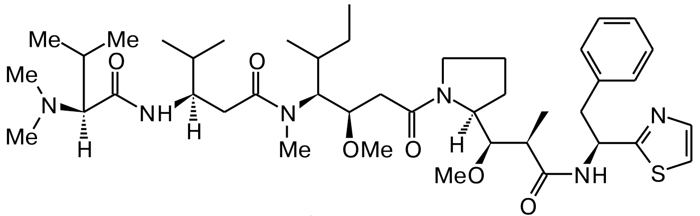
84

Compound **80** contains a rare monosaccharide 6-deoxytalosamine (pneumosamine) and polysaccharide **81** incorporates a sulfate group, which has not been found previously in antigenic polysaccharides from gram-negative bacteria. L-Iduronic acid was found in polysaccharide **82**, polysaccharide **83** is a phosphate-containing compound incorporating a number of rare 6-deoxysugars, while **84** contains 2-amino-2-deoxygycerol residues (GroN).

Generally, marine biopolymers have been less studied than the corresponding low-molecular-weight bioregulators, although they are of interest for biotechnology. This is especially true for substances such as enzymes from marine macro- and microorganisms and peptide hormones of marine animals including fishes.

Practical use of the results of investigations in marine bioorganic chemistry

The efforts of scientists from several countries have resulted in determination of the structures and investigation into the properties of a whole series of marine natural products including those presenting substantial interest for medicine due to their exceptionally high physiological activity. The attention was focused on highly active antitumor substances of the marine origin.[127] These include the peptides dolastatins, for example, dolastatin 10 (**85**) from the mollusc *Dolabella auricularia*, which (taken in a dose of 11 μg kg^{-1}) ensured survival of 80% laboratory animals with melanoma B16, macrolides spongistatins, for example, spongistatin-1 (**86**), which proved to be the most active of all the natural and synthetic products studied at the National Cancer Institute (USA). The alkaloid ET-743 (**87**) appears to be the first of the new generation of antitumor drugs developed on the basis of marine secondary metabolites. This substance, isolated from the Caribbean ascidium *Ecteinascidia turbinata*,[128] is the active substance of a drug developed by the Pharmamar company (Spain) and used in Europe to treat sarcomas. A series of other marine antitumor natural products, for example, bryostatin-1 (**88**) from the bryozoan *Begula nerita*, the above-mentioned dolastatin-10, and aplydin (**89**) from the ascidian *Aplydium albicum* are under the final stages of clinical trials.[127,129–131]



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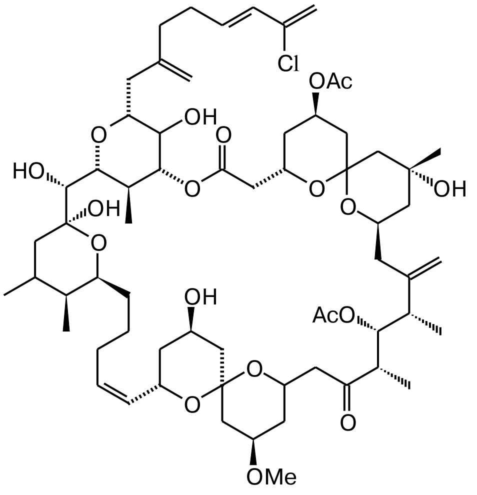
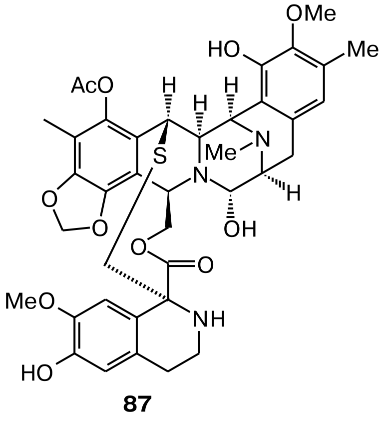
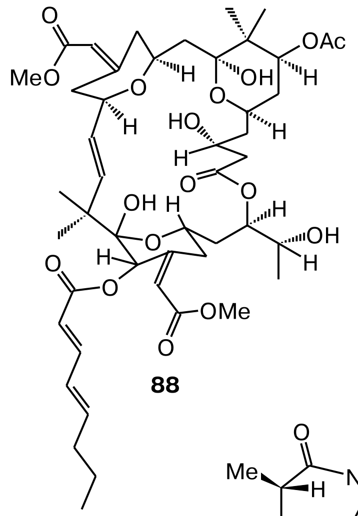


86



87



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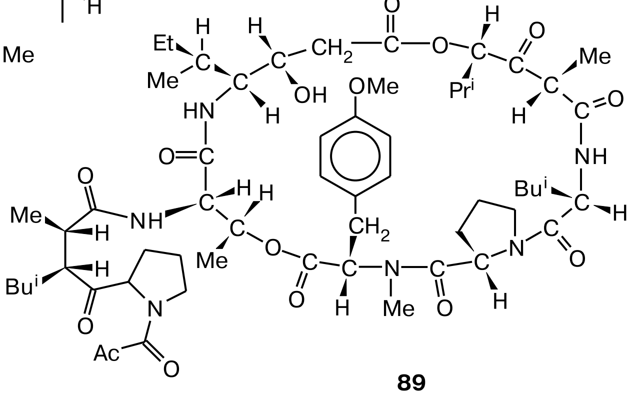

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The studies carried out at the PIBOC were focused on the marine natural products exhibiting antioxidant, antiburn, and immunostimulating properties. On the basis of the low-molecular-weight compounds and biopolymers studied, a series of medical products, veterinary preparations, prophylactic drugs, and food additives have been developed and have found practical application.

A complex of collagenolytic proteases from the hepatopancreas of the king crab (waste from its processing) has been used to develop a new drug, "Collagenase KK",[132] which has now been certified in Russia and is used for enzymatic cleaning of wounds with ulceration and necrosis including burns, frostbites, gangrenes, chronic osteitis, varicose ulcers, *etc*. "Collagenase KK" and the synthetic sorbent "Gelevin" are components of a new dressing remedy "Collavin". When "Collavin" is applied onto wounds, it swells while sorbing and draining the wound fluid from the wound floor to the outside, which favors fast healing of wounds with large amounts of necrotic tissues.[133]

Pigments of sea urchins possessing antioxidant, antimicrobial, and antiinflammatory properties have served as the basis for new drugs now certified in Russia, called "Histochrom for ophtalmology" and "Histochrom for cardiology".[134,135] The former demonstrated high efficiency in the therapy of hemophthalms, burns, and some other eye diseases, while the latter is a cardioprotector decreasing the necrosis area at acute cardiac infarction.

The veterinary drug "KD", based on immunostimulating triterpene glycosides from the commercial holothurian *Cucumaria japonica*, has been successfully employed for many years in fur farming to treat and prevent the aleutian disease of minks. This is also useful in reducing the mortality from infectious diseases in pigs, dogs, and other animals.[136]

"Zosterin",[137] a polysaccharide drug from sea grasses, which is being produced industrially since 1986, is a highly active polyanionic sorbent that binds heavy metal atoms and removes them from the organism and exhibits hypocholesteremic, antibacterial, antiulcer, and antiallergic actions. "Zosterin" is a component of a number of prophylactic drugs, drinks, and other industrial foodstuffs.

"Mytilan",[138] a carbohydrate—protein complex from wastes from processing of the mussel *Crenomytilis grayanus* is used successfully as a biologically active additive and a hydrating component in the cosmetic creams produced by pharmaceutical industry (*e.g.*, Linda and Svoboda factories, *etc.*).

Nonalcoholic prophylactic balsams of the "Herbamarin" series[139] (four sorts of these balsams have now been developed), which are produced by the joint-stock company Ussuriisk Balzam, contains extracts from medicinal herbs certified for use by the Pharmacopeia of Russia and biological preparations from edible marine species. Various balsams of this series exhibit tonic, hepatoprotecting, cardioprotecting, and antidiabetic action.

Thus, the fundamental research of the structure, biogenesis, and physiological activity of new metabolites of the marine origin has created a sound base for the development and subsequent clinical application of new medicinals, prophylactic drugs, and other biopreparations. Both Russian and foreign scientists faced a number of difficulties and problems. In many cases, bioactive marine low-molecular-weight substances are produced by rare and dispersed species, whose collection could hardly be arranged and whose withdrawal on an industrial scale would cause irreversible damage to the biological diversity due to extinction of the corresponding marine animals or plants. This problem is especially acute in relation to antitumor substances, which often occur in the producers in minor quantities. In these cases, the solution of this problem rests on either of two approaches, namely, mariculturing of the producer or synthesis of the target compound. For example, the culturing was used to solve the problem of the starting material for the production of ecteinascidine ET-743. For this purpose, huge plantations for growing the ascidian *Ecteinascidia turbinata* at the coast of Spain were formed. For clinical trials of dolastatin-10, methods for its total synthesis have been developed and more than 15 g of this substance has been produced. With the exceptionally high activity of this peptide, this amount is quite sufficient.

The synthetic approach is widely used at our Institute; the active substance of the "Histochrom" drugs,[140,141] the antitumor natural products fascaplisin,[142] polycarpin,[31] and a number of other marine metabolites have been synthesized.

The manufacture of biopreparations based on marine biopolymers is often hampered by the absence of a reliable source of raw materials and by the properties of preparations themselves, in particular, by low stability. The use of fishery wastes as the raw materials provides the possibility of the corresponding biotechnological process. The problem was solved in this way in the case of "Collagenase KK", "Mytilan", and "KD" preparations.

The approach based on the use of microbial biotechnology is also promising. The only Russian collection of marine microorganisms (the acronym is KMM), which is a part of the World Federation Culture Collection, is deposited at our Institute. Some axenic* strains of bacteria and fungi from this collection are perfect producers of various enzymes and other biologically active substances.[143—145]

Finally, the genetic-engineering approach opens up broad opportunities for the creation of microbial superproducer strains and cultures of marine organism cells and tissues for the preparation of valuable substances. Investigations along this line of marine biotechnology are in progress in a number of countries including Russia.

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* The term axenic means purified from other microorganisms.

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