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New trends of marine biotechnology development

G.B.Elyakov, T.A.Kuznetsova, V.A.Stonik, V.V.Mikhailov

Pacific Institute of Bioorganic Chemistry, Far East Division, Russian Academy of Sciences, 690022, Vladivostok, Russia

ABSTRACT

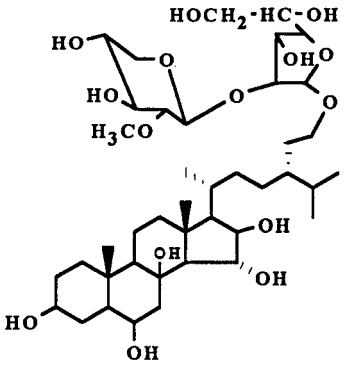
The present paper deals with prospects of marine biotechnology development, including biotechnological applications of enzymes from marine invertebrates and utilizations of marine microorganisms for the obtaining both low and high molecular weight natural products.

INTRODUCTION

For the last years chemistry of marine natural products has turned into a fast growing field of life sciences. Many marine metabolites attract more and more attention due to their high physiological activities and great significance for intra- and interspecies communications in biocenoses. Recently marine biotechnology has come up on the achievements of marine natural products chemistry. In this report we would like to show some interrelations between these two directions of the studies on marine organisms, using as examples mainly some last works of the Pacific Institute of Bioorganic Chemistry (Far East Division of the Russian Academy of Sciences, Vladivostok).

FROM STUDIES ON MARINE NATURAL PRODUCTS TO ENZYMATIC TRANSFORMATIONS OF SOME SUBSTRATES

Microbial transformations of steroids are the well-documented biotechnological processes, utilized for purposes of steroid drugs industry. However, in a steroid nucleus there are numerous positions which are impossible for introducing functional groups by this method. On the other hand, the results of chemical investigation of marine invertebrates, especially echinoderms, have demonstrated a great variety of the positions, oxidated during the biosynthesis of some steroid metabolites (Ref.1-3). Starfish were also shown to be as sources of steroid polyols, polyol sulfates and glycosides with very high level of oxidation in steroid moiety. Recently new steroid biosides (1,2) have been isolated from the Far-Eastern starfish *Crossaster papposus (Ref.4)* . These compounds have the aglycones, oxidated at positions 3,6,8,15,16,29. Steroid polyols (3-5) and glycosides (6,7) from Far-Eastern species can be considered as other examples, demonstrating the possibilities of the oxidation (Ref.5,6).



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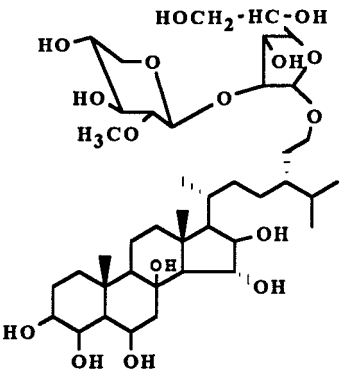

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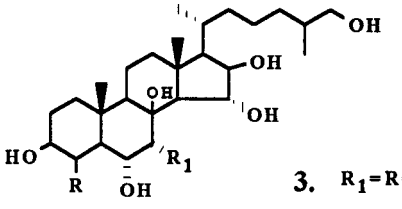
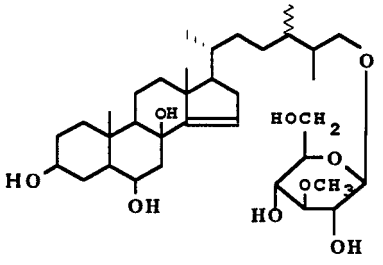


3. R_1=R=OH

4. R_1=OH, R=H

5. R_1=R=H



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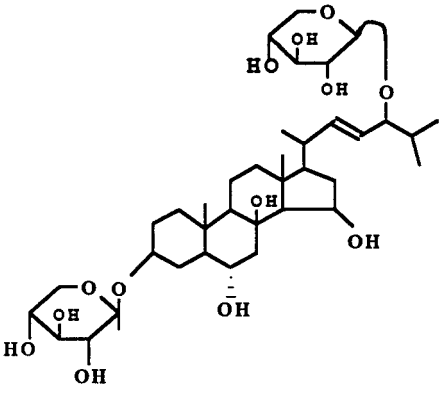

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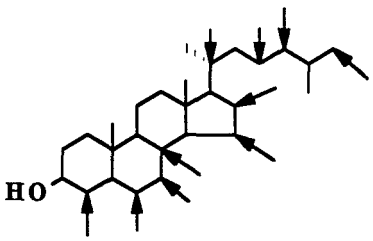
If we take into consideration all isolated steroid metabolites, it may be concluded that in starfish there is a series of enzymes, oxidating cholestane, ergostane and stigmastane sterols. Positions of such oxidations are given below and marked by arrows.



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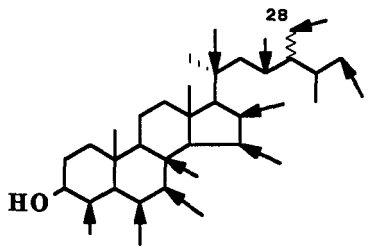

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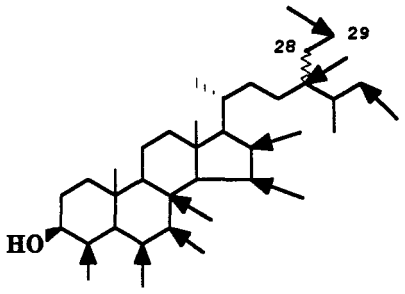

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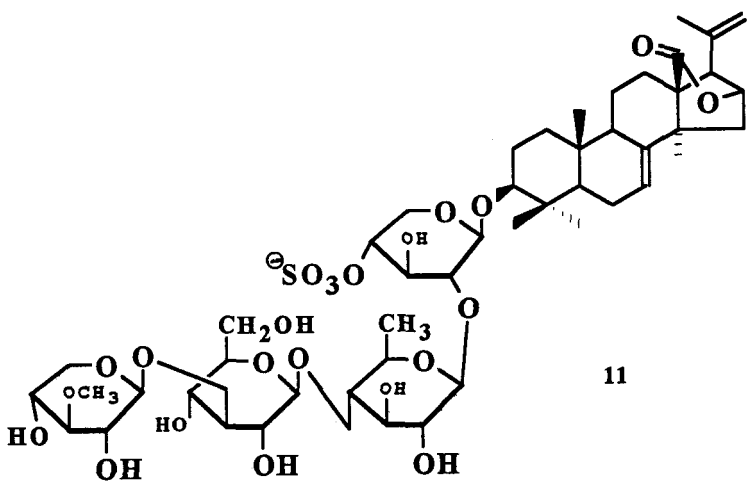
Other echinoderms also contain enzymes, oxidating steroid and triterpenoid precursors. For example, sea cucumbers obviously metabolize lanosterol into aglycones of glycosides with oxidative lose of a part of side chains. Several new types of glycosides, which are represented ,for example, by cucumarioside G_2 (11) from *Eupentacta fraudatrix* and kuriloside A (12) from *Duasmodactyla kurilensis* have been isolated (Ref.7,8).



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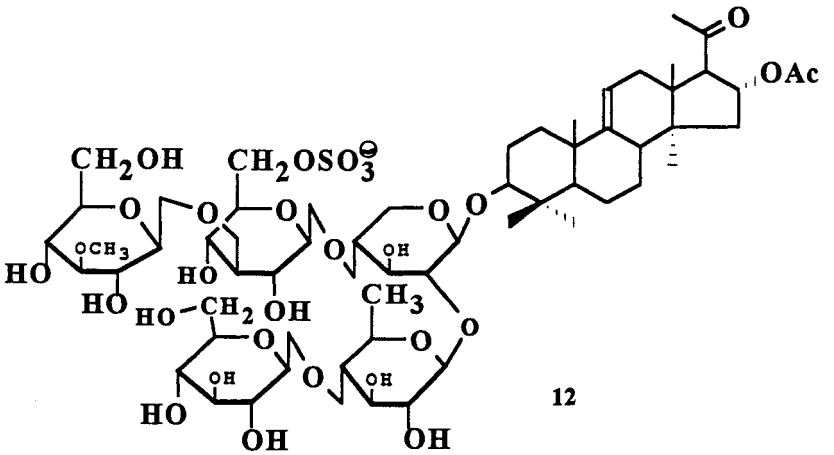

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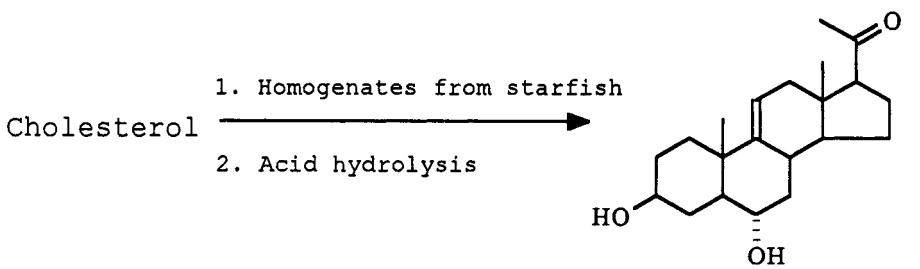
To demonstrate the principal possibility to use enzymatic systems of echinoderms for biotechnology we have shown that homogenates from various tissues of starfish can transform cholesterol into asterosaponines. These glycosides yield so-called asterone (13) after acid hydrolysis (Ref.9). Asterone can be as an intermediate in chemical synthesis of corticosteroids.



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MARINE MICROORGANISMS AS PRODUCERS OF PHYSIOLOGICALLY ACTIVE SUBSTANCES.

The investigation of marine microorganisms associated with marine macroorganisms is a rather new trend of marine biotechnology. Such microorganisms have been found to be producers of many well-known marine toxins including tetrodotoxin (Ref.10), saxitoxin (Ref. 11), and prosurogatoxin (Ref. 12,13). Moreover, studies on metabolites of the marine microorganisms-associates let us reveal and regulate interrelations between biological components of marine biosystems. On the other side, there is an opportunity to use these microorganisms to obtain important physiologically active substances (PAS). It should be taken into attention that such approach can help also to solve very significant ecological problems. In 1985 at the Pacific Institute of Bioorganic Chemistry the study on metabolites of marine microorganisms from various marine sources were begun. A collection of marine microorganisms from torrid and boreal zones of the World Oceans was created. Its official acronym in the World

Federation for Culture Collection is KMM. This collection is a basis to study taxonomy of marine microorganisms and biosyntheses of PAS.

Ability of marine bacteria to synthesize superbrominated compounds was detected earlier. We isolated several strains of *Vibrio spp.* from the sponge *Dysidea sp.* that synthesized cytotoxic and antibacterial tetrabromodiphenyl ethers (Ref.14,15). One of these metabolites 3,5-dibromo-2-(3',5'-dibromo-2'-methoxy-phenoxy)-phenol was earlier isolated from the sponge itself. Not always we could find a relation between metabolites of bacteria-associates and the substances isolated from macroorganisms. So, isocoumarin antibiotics were not described from the sponge *Dendrilla sp.*, but bacterial strains *Bacillus pumilus* (D-7 and D-12), associated with the sponge produced these compounds. Two from them were identical to amicoumacins B and F from the terrestrial strain *B.pumilus* (A1-77) (Ref.16). The compounds were interesting due to their antidepressive activity and the ability to inhibit the growth of the plant pathogenic microorganism *Xanthomonas bardii.*

Studying toxin-producing bacteria from soft corals *Palythoa spp.*, we found bacterial toxins with antigenic specificity similar to palytoxin but differing from this toxin in some biochemical characteristics. These data allowed us to confirm that we dealt with palytoxin-like bacterial metabolites. Besides, the bacterium *Bacillus sp.* (KMM 456), associated with some species of *Palythoa* produced cytotoxic substances. They has high pH-dependant cytotoxic activity against Ehrlich carcinoma cells and mouse erythrocytes. The complex of the cytotoxins contains five peptides, a main of those has the molecular weight of 1049. All the substances have the identical amino acid composition: valine, leucine, aspartic and glutamic acids in the ratio of 1:4:1:1. Mass-spectrometry and NMR C^{13} spectroscopy showed the peptides to contain also non-amino acid components. The peptide structures are under way.

Other peptide metabolites with high antifungal activities were characteristic for the isolates from *Bacillus sp.*(KMM 457), associated with the soft coral *Sarcophyton sp.* These non-toxic small peptides consists of threonine, serine, glutamic and aspartic acids, proline, tyrosine. Their structural investigation are also in process.

New cyclic depsipeptides with pH-dependant cytotoxic activity against tumor cells were found in the culture of *B.pumilus* (KMM 150), associated with the Australian sponge *Ircinia sp.*. Their structural studying were done by us together with Anne Wood (University of Puget Sound, Tacoma, USA), Peter Murphy and Rick Willis (Australian Institute of Marine Science, Townsville, Australia). We determined that the cytotoxic activity of the peptides was bound up with the membranolytic effect. That increased in mild acidic medium and decreased in the presence of bovine albumin *in vitro*. For the last years high-active small peptides, including cyclic depsipeptides, have been isolated from sponges. However, a question about genuine producers of such compounds have not been discussing in literature. At the same time we showed similar cyclic depsipeptides to be biosynthesized by bacteria-associates.

The first systematic research of the marine microorganism metabilites were concerned to establishing chemical structure of antibiotics from marine actynomycetes. Significant interest of researchers in marine *Actinomycetales* was caused by the fact that terrestrial representatives of these microorganisms have given a great number of PAS. A series of works reported the actinomycetes, isolated from marine substrates, to differ from terrestrial organisms of the same geographical zones in their phenotypic features. In particularly, they had halo- and barotolerance (Ref.17,18), hydrolyzed agar, alginate, chitin, laminarin (Ref.19,20). Based on these facts, we have done the following conclusion: for a long time representatives of actinomycetes have been inhabiting marine substrates and they are active members of marine microorganism associations. That is why, the search for PAS, produced by new forms of actinomycetes, especially belonging to rare and undescribed taxa is promising. Recent data on microbiological studies have allowed suggesting the existence of such forms into marine ecosystems.

Our investigation concern actinomycetes of marine sediments from various depths and various geographical zones of the World Ocean. More than 150 strains of marine actinomycetes of genera *Nocardia, Streptomyces, Micromonospora* and *Promicromonospora* have became as the subjects of our chemical and biochemical studies. In the present review we would like to report a new protein antibiotic - palmyromycin. The latter was isolated from the actinomycete *Streptomyces pluricolorescens* (KMM Acl). This compound did not show cytotoxic and proteolytic activities. The antibiotic consists of five subunits of 18 KD molecular weight. Its MIC against *Stuphylococcus aureus* and *Bacillus subtilis* is of 0.1-1.0 mkg/ml (Ref.21).

We have studied antiviral activity of the bacteria living in animals, sea water and sediments of the Great Barrier Reef (Ref.22). It was shown that 72 strains from 535 (13.5%) inhibited thymidinekinase. These were the strains from sponges (13.3%), bottom sediments (26.5%), ascidians (17.6%), coelenterates (14.6%), sea water (10.6%). Metabolites of 16 strains from 224 appeared to be effective to reverse transcriptase. The largest number of producers of such inhibitors (33.3%) was found in microorganisms from sea water. Twelve strains from 224 inhibited RNA-polymerase. Especially many such strains were isolated from coelenterates.

MARINE MICROORGANISMS AS SOURCES OF HIGH MOLECULAR WEIGHT COMPOUNDS

We observed some specificity in the interrelation between micro- and macroorganisms. So, we isolated two lectins from the mussel *Crenomytilus grayanus*. These lectins interacted with the bacteria, associated with the host organism and did not agglutinate many human and animal pathogene bacteria. One of the lectins interacted exclusively with Gram-positive bacteria, another one was more effective to Gram-negative bacteria (Ref.23). These data show an

animal-host can select microorganisms-associates with specific properties.

Marine microorganisms may be used as sources for industrial production of many enzymes. Such enzymes as carrageenases, endo-β-1,3-xylanase, proteinases, thermostable alkaline phosphatase are known to be isolated from marine microorganisms. An enzyme from *Pseusomonas sp.* demonstrated the maximal activity in NaCl solution at concentration of 18% and was suitable for producing fish sauces (Ref.24).

We have isolated a strain *Alteromonas macleodii* (KMM 162) from the mussel *Crenomytilus grayanus* (Peter the Great Bay, Japan Sea), which produces a high-active alkaline phosphatase (7,000 units per 1 mg of substrate) (Ref.25). Its activity is in a range of pH 7.5-11.0 with the maximum at pH 9.5-9.8 at the presense of 1 mM $MgCl_2$. The phosphatase was thermostable, but at the presence of some sulphohydryl reagents it might be inactivated at 60° C for 10 min. A method to produce simultaneously the alkaline phosphatase and endo-β-1,3-glucanase was developed. By the method, addition of laminarin into a nutrient medium gave a high yield of both the enzymes. Recently we have isolated a bacterium, perhaps a new species of *Alteromonas*, producing an alkaline phosphatase with activity of 10,000 units per 1 mg of substrate.

To find new bacterial strains, secreting elastolytic enzymes, we treated 278 various cultures. So, strains *Kurthia sp.* were isolated from the sponge inhabited the waters of Kuril Islands. These strains produced an elastase into the culture broth (150 mg of the crude enzyme per 1l of culture broth) (Ref.24). A strain *Alteromonas haloplanktis* (KMM 223), isolated by us from a sea water sample of 2000 m depth (Noth-Western part of the Pacific Ocean) in 1985, produced an unique enzyme - uridin-specific RNAse. It consists of two identical polypeptide chains with the molecular weight of 26 KD each, and has pH optimum at 8.5. A strain *Bacillus pumilus* (KMM 61) from the sponge *Dendrilla sp.* (Madagascar) synthesized intracellular RNAse of 14 KD molecular weight. This enzyme is active at pH 8.5-9.5. It lost its activity at the absence of NaCl. This RNAse hydrolyzed purine and pyrimidine homopolyribonucleotides. Then an intercellular RNAse with 12.5 KD molecular weight and maximal activity at the same pH range was isolated from the bacterium *B.pumilus* (KMM 62) that in its turn, was found in the sponge *Suberites sp.* (Seyshelles). The enzyme splited substrates by nuclease mechanism to form oligonucleosides with 3'-terminal phosphate groups and various length of the chains. Hydrolyzing polynucleosides, the RNAse showed purine specificity (Ref.23).

New restrictases were isolated from *Vibrio nereis* (VneI) and *Vibrio sp.* (VspI) (Ref.26,27). Restrictase VneI recognized sequence (5')GT TAAT. These enzymes are not isoshisomeric to known enzymes. Recently we found *Alteromonas spp.* (maybe several new species of the genus). They produce α-galactosidases, tyrosinases and carrageenases with good yields. The search for new bacterial producers of these enzymes was undertaken because of its great significance.

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