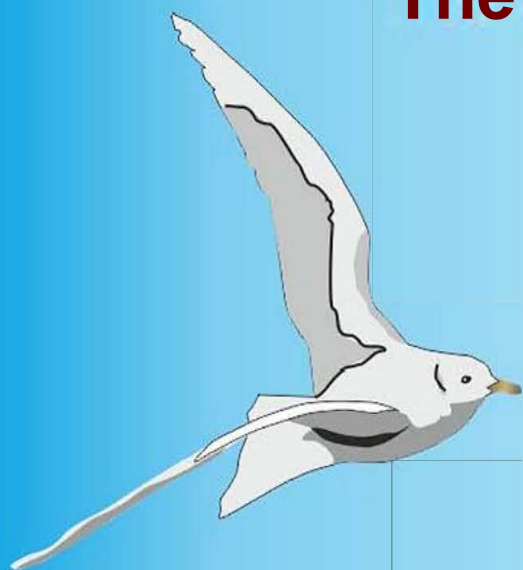


The 3rd International Symposium on Life Sciences



**September 4-8, 2018
Vladivostok, RUSSIA**

Proceeding & Abstracts



On **September 4 - 8, 2018** in Vladivostok (Russia), G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences (PIBOC FEB RAS) organizes

THE 3rd INTERNATIONAL SYMPOSIUM ON LIFE SCIENCES

Topics are structured in three sessions:

- **Natural Products**
- **Biotechnology**
- **Molecular Immunology**

Satellite Symposium **“KORUS-2018”** is also held.

Organizing Committee:

Valentin Stonik	- Chairman, PIBOC FEB RAS, Russia
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Symposium Secretariat

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The symposium was held with the financial support of: Russian Foundation for Basic Research (project No. 18-04-20060)





Dear Colleagues and Friends:

It is my pleasure and honour to heartily welcome you in Vladivostok for the 3rd International Symposium on Life Sciences. Five years have already passed since we met in 2013 at our second Symposium. Since then the relationships with our colleagues have strengthen. We believe scientific community all over the World to be the most solid one as we share common interests in obtaining new knowledge about nature and human society.

For 54 years our Institute, s named after its founder G. B. Elyakov, has been engaged in the studies of marine and terrestrial natural products including collection and identification of natural objects to search for new sources of biologically active metabolites, as well as in the isolation of these compounds, elucidation of their structure, and development of new medical preparations. The investigations of marine natural products have become a main trend.

We hope that our meeting will be fruitful and all the participants will be interested in the presentations of their colleagues. The Scientific program was planned to provide a broad overview of the investigation fields of natural products: biotechnology, enzymology, molecular biology, and genetic studies.

Taking into attention that Vladivostok and Primorsky Region have a rich history and culture, we plan to represent their peculiarities in our Social program. Vladivostok is the capital city of Primorskiy Krai and the largest port in the Russian Pacific, the terminal of the Trans-Siberian railroad. The city founded in 1860 is rather young, but plays an important role in the Russian history, economy, defense, and military construction. At present Vladivostok is large well-recognized Pacific center of culture, education, and science. A dozen of Institutions belonging to the Russian Academy of Sciences are now located in Vladivostok and carry out scientific investigation in biology, chemistry, physics, mathematics, and many other sciences. Russian scientists collaborate successfully with their colleagues from foreign countries, especially from Asia, for the studies on different aspects of life sciences.

In the conclusion, we would like to thank our sponsors for their financial support in arranging this Symposium.

Thank you for attending.

A handwritten signature in blue ink, appearing to read 'Stonik', with a long, sweeping underline.

*Prof. Valentin Stonik
Chairman of the Organizing Committee
Scientific Leader of PIBOC*

THE 3RD INTERNATIONAL SYMPOSIUM ON LIFE SCIENCES

September 4-8, 2018, Vladivostok, Russia

September 4

10:00 – 17:00 **Arrival and Accommodation**
17:00 – 19:00 **Welcome party**

SCIENTIFIC PROGRAM

September 5

09:30 – 10:00 **Registration of participants**
10:00 – 11:00 **Welcome addresses**

Session 1: NATURAL PRODUCTS

CHAIRMEN: Sergey Kozlov

- 11:00 – 11:30** **Tatiana Makarieva**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Search and structural studies of secondary metabolites from Far Eastern marine invertebrates
- 11:30 – 11:45** **Pavel Dmitrenok**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Metabolomic approaches in the studies of holothurian and starfish glycosides
- 11:45 – 12:00** **Elena Leychenko**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
The combinatorial library of actinoporins from the sea anemone *Heteractis crispata*
- 12:00 – 12:15** **Galina Melman**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Reactions of polyhydroxynaphthazarins and their methyl ethers with aqueous ammonia
- 12:15 – 12:30** **Dmitry Farmakovskiy**
Shimadzu Europa GmbH, Moscow, Russia
Shimadzu's new culture media analyzer platform for fundamental research and process development in cell cultures
- 12:30 – 13:30** **Coffee break & Poster session**
- 13:00 – 13:15** **Irina Bakunina**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Slow-binding irreversible inhibitors of recombinant alpha-galactosidase from marine bacteria *Pseudoalteromonas* sp. KMM 701 and its C494N mutant
- 13:15 – 13:30** **Ekaterina Lyakhova**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
The studies on structures and absolute stereochemistry of secondary metabolites using various modern approaches
- 13:30 – 13:45** **Alexandra Silchenko**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Structural diversity and some biosynthetic peculiarities of triterpene glycosides from the sea cucumbers

13:45 – 14:00 **Kseniya Tabakmakher**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
 Structural studies of pentacyclic guanidine alkaloids from the Far Eastern marine sponge *Monanchora pulchra*

14:00 – 15:00 **Lunch**

Session 2: BIOTECHNOLOGY

CHAIRMEN: Dmitriy Aminin

15:00 – 15:30 **Rita Bernhardt**

Saarland University, Germany

Bacterial cytochromes P450 are highly efficient and promising terpene hydroxylases

15:30 – 15:45 **Marina Isaeva**

G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia

Molecular cloning of sea cucumber oxidosqualene cyclases

15:45 – 16:00 **Larisa Balabanova**

G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia

Marine bacterial enzymes for molecular genetics and structure-function studies

16:00 – 16:15 **Evgenia Bystritskaya**

G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia

Genomic approach to the search for enzymes for triterpene glycoside transformation

16:15 – 16:30 **Elena Leshchenko**

G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia

Seagrass-derived fungi as a source of bioactive compounds

16:30 – 17:00 **Coffee break & Poster session**

17:00 – 17:15 **Nina Buinovskaya**

G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia

Dephosphorylation of lipopolysaccharides by alkaline phosphatase from marine bacterium

17:15 – 17:30 **Nadezhda Chernysheva**

G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia

Comparative genomics of *Zobellia*: analysis of polysaccharide lyases genes and operons

17:30 – 17:45 **Elena Ivanets**

G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia

Auroglaucin and echinulin related metabolites from vietnamese marine sediment-derived fungus *Eurotium niveoglaucum*

September 6

Session 1: NATURAL PRODUCTS

CHAIRMEN: Pavel Dmitrenok

9:30 – 10:00 **Igor Kasheverov**

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia

Oligo-arginine peptides as a new class of ligands of nicotinic acetylcholine receptors

10:00 – 10:15 **Irina Agafonova**

G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia

Protective properties of Histochrome in experimental stroke brain models

- 10:15 – 10:30 **Irina Gladkikh**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
First two-chain peptide toxin from sea anemone *Heteractis crispa*
- 10:30 – 10:45 **Anton Yurchenko**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
The secondary metabolites from the marine-derived fungus *Aspergillus flocculosus*
- 10:45 – 11:00 **Aleksandra Kvetkina**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
The new *Heteractis magnifica* Kunitz-peptide interacts with serine
- 11:00 – 11:30 **Coffee break & Poster session**
- 11:30 – 11:45 **Lee-Chiang Lo**
Department of Chemistry, National Taiwan University, Taiwan
Development of chemical probes exploiting quinone methide chemistry for biochemical applications
- 11:45 – 12:00 **Sergey Kozlov**
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia
The development and characterisation of novel ligands to ASICs
- 12:00 – 12:15 **Galina Likhatskaya**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Structural bioinformatics in the study of cold-active enzymes from marine organisms
- 12:15 – 12:30 **Rimma Kalina**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
The first peptide ASIC1a channel modulators from sea anemone
- 12:30 – 12:45 **Oksana Sintsova**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
 α -Amylase inhibitors are major components of sea anemone *Heteractis magnifica* mucus
- 12:45 – 13:00 **Alexey Belik**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Properties and substrate specificities of alginate lyases from marine bacterium *Formosa algae* KMM 3553

13:00 – 14:00 **Lunch**

Session 3: MOLECULAR IMMUNOLOGY

CHAIRMEN: Kuo-Feng Hua

- 14:00 – 14:30 **Ingolf Bernhardt**
Saarland University, Germany
Red blood cells: ion transport, role in thrombus formation, and interaction with artificial surfaces and nanoparticles
- 14:30 – 14:45 **Yun-Ming Wang**
National Chiao Tung University, Taiwan
Activation of angiogenesis and wound healing in diabetic mice using \bullet NO-releasing dinitrosyl iron complexes
- 14:45 – 15:00 **Alexey Ivanov**
Institute of Biomedical Chemistry, Moscow, Russia
SPR biosensors in direct molecular fishing: implications for protein interactomics

- 15:00 – 15:15 **Ekaterina Sokolova**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Influence of red algal polysaccharides on neutrophils activation, cytokine synthesis and intestinal epithelial cells *in vitro*
- 15:15 – 15:30 **Anna Zakirova**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Electrocardiography study of diglutathionyl analog of echinochrome on adrenaline-induced myocardial ischemia in mice
- 15:30 – 16:00 **Coffee break & Poster session**
- 16:00 - 16:15 **Tian-Lu Cheng**
Kaohsiung Medical University, Taiwan
Autologous hinge as a universal antibody lock enhance the selectivity and safety of antibody drug
- 16:15 – 16:30 **Olga Tarakova**
Far Eastern Federal University, Vladivostok, Russia
Cationic hybrid nanoparticles for cancer visualization and therapy
- 16:30 – 16:45 **Rita Chen**
Institute of Biological Chemistry, Academia Sinica, Taiwan
Peptide therapy for the prevention of Alzheimer's disease
- 16:45 – 17:00 **Woochul Chang**
Pusan National University, South Korea
Therapeutic application of MSC-derived exosome
- 17:00 – 18:00 **Coffee break & Poster session**
- 18:00 – 22:00 CITY TOUR**

September 7

Session3: MOLECULAR IMMUNOLOGY

- CHAIRMEN: Alexey Ivanov**
- 9:30 – 10:00 **Kuo-Feng Hua**
National Ilan University, Taiwan
Therapeutic targeting of NLRP3 inflammasomes by natural products
- 10:00 – 10:15 **Chien-Chih Yang**
National Taiwan University, Taiwan
The roles of a transcription factor ICE1 in plant ABA-dependent signaling pathways
- 10:15 – 10:30 **Alexandra Seytkalieva**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Enzymatic bioassays used for pollution monitoring of marine environment
- 10:30 – 10:45 **Georgy Nevinsky**
Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
Mechanisms of autoimmune diseases development
- 10:45 – 11:00 **Ekaterina Yurchenko**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Neuroprotective activity of marine fungi metabolites in toxin-induced model of Parkinson's disease
- 11:00 – 11:15 **Maxim Kokoulin**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Sulfated lipopolysaccharides from marine gram-negative bacteria: structure and biological activity
- 11:15 – 11:45 **Coffee break & Poster session**

KORUS-2018

CHAIRMEN: Valentin Stonik

- 11:45 – 12:00 **Hyoung-Kyu Kim**
Cardiovascular and Metabolic Disease Center, Inje University, South Korea
A novel atypical PKC- ι inhibitor, echinochrome A, enhances cardiomyocyte differentiation from mouse embryonic stem cells
- 12:00 – 12:15 **Jin Han**
Inje University, South Korea
Physiological role of AMPK modulatory protein in heart and mitochondria
- 12:15 – 12:30 **Jong-Young Kwak**
Pioneer Research Center, School of Medicine, Ajou University, South Korea
Prolonged three dimensional culture of primary hepatocytes for drug metabolism analysis
- 12:30 – 12:45 **Hyung-Sik Kim**
Pusan National University, South Korea
Stem cell-based basic to translational research for immune disorders: toward the establishment of cellular platform for the screening of marine natural products
- 12:45 – 13:00 **Hwayoung Yun**
Pusan National University, South Korea
Unified and protecting-group-free total synthesis of natural indene sesquiterpenoids and their derivatives
- 13:00 – 14:00 **Lunch**
- 14:00 – 14:15 **Nari Kim**
Inje University, South Korea
Cardiovascular remodeling in diabetic atherosclerosis
- 14:15 – 14:30 **Jong-Ki Kim**
School of Medicine, Catholic University of Daegu, South Korea
Coulomb nanoradiator-mediated, site-specific thrombolytic proton treatment with fucoidan-conjugated magnetite
- 14:30 – 14:45 **Jee-Yeong Jeong**
Kosin University College of Medicine, South Korea
Erythropoietin (EPO) receptor expression and the effects of EPO on diffuse large B cell lymphomas
- 14:45 – 15:00 **Sergey Fedoreyev**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Antiviral activity of histochrome and antioxidant composition containing echinochrome
- 15:00 – 15:15 **Sang Hong Baek**
The Catholic University of Korea School of Medicine, South Korea
Pivotal cytoprotective mediators and promising therapeutic strategies for EPC-Based cardiovascular regeneration
- 15:15 – 15:30 **Elena Vasileva**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Development of new drug forms based on echinochrome a using electrospun micro/nanofibers
- 15:30 – 15:45 **Darya Tarbeeva**
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Prenylated polyphenolic compounds from *Lespedeza bicolor*
- 15:45 – 16:00 **Coffee break**
- 16:00 – 16:30 **Summarizing remarks**

17:00 – 20:00 CLOSING PARTY

LIST OF POSTERS

- 1 **Valerii Surits**, R. Usoltseva, N. Shevchenko, S. Ermakova
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
The structural characteristics and anticancer activity of native and modified fucoidans from *Sargassum duplicatum* and *Sargassum feldmannii*.
- 2 **Natalia Mishchenko**, S. Fedoreyev, E. Vasileva, N. Krasovskaya
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Study of the echinochrome stability and the products of its oxidative transformation
- 3 **Gennadii Nabereznykh**, S.I. Bakholdina, V.N. Davydova, T.F. Solov'eva
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Chitosan and its acyl derivatives included in liposomes protect mice against endotoxin shock
- 4 **Anastasiia Zueva**, A.S. Silchenko, S.P. Ermakova
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Study of substrate specificity of two recombinant fucoidanase from marine bacteria *Wenyngzhuangia fucanilytica*
- 5 **Yulia Dubrovskaya**, T.N. Makarieva, L.K. Shubina, I.Yu. Bakunina
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Effect of pentacyclic guanidine alkaloids on activity of natural β -1,3-D-glucanase from marine hydrobionts
- 6 **Kristina Belokozova**, O.S. Malyarenko, S. D. Anastyuk
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Mass spectrometry of sulfated laminaran derivatives, obtained by autohydrolysis in heavy-oxygen water
- 7 **Alexev Belik**, K.M. Tabakmakher, T.N. Makarieva, T.N. Zvyagintseva, S.P. Ermakova
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Modes of action of sulfated steroids on recombinant endo-1,3- β -D-glucanase and alginate lyase from marine bacterium *Formosa algae* KMM 3553
- 8 **Evgeniy Pislyagin**, E.S. Menchinskaya, O.F. Smetanina, E.A. Yurchenko
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Influence of same marine fungi metabolites on reactive oxygen species level in neuroblastoma cells
- 9 **Ekaterina Menchinskaya**, S.A. Dyshlovoy, E.V. Ivanets, E.A. Yurchenko
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Cytotoxicity of same marine fungi metabolites against cancer cells
- 10 **Antonina Klimenko**, D.V. Tarbeeva, A.S. Blagodatski, P.G. Gorovoy, S.A. Fedoreyev
Far Eastern Federal University, Vladivostok, Russia
Polyphenolic compounds from *Ampelopsis japonica* inhibit Wnt signaling
- 11 **Anna Kaspruk**, R.V. Usoltseva, N.M. Shevchenko, S.P. Ermakova
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
The fucoidans from brown algae *Saccharina cichorioides*, *Saccharina japonica* and *Laminaria longipes*
- 12 **Yuliva Noskova**, L.A. Balabanova, N.A. Terentieva
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Alkaline phosphatase / phosphodiesterase from marine bacterium *Cobetia amphilecti* KMM 296

- 13 **Evgeniy Sidorin**, V.A. Khomenko, T.F. Solov'eva
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
The effect of pH on the chaperone activity of Skp from *Yersinia pseudotuberculosis*
- 14 **Valentina Khomenko**, E.V. Sidorin, S.I. Bakholdina, N.U. Chernysheva, N.U. Kim, M.P. Isaeva, T.F. Solov'eva
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
The culture temperature affects the properties of the *Yersinia pseudotuberculosis* porin inclusion bodies and the structure of the recombinant porin
- 15 **Alexandr Menshov**, E.A. Zelepuga
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
APETx-like peptides interaction with ASIC channels: comparative *in silico* study
- 16 **Sergey Kozlovskii**, O. Sintsova, I. Kasheverov, Y. Korolkova, I. Mosharova, S. Koshelev, E. Yurchenko, S. Kozlov, E. Leychenko
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
First steps in studying of jellyfish *Gonionemus vertens* venom
- 17 Natalya V. Ageenko, Konstantin V. Kiselev, **Nelly A. Odintsova**
National Scientific Center of Marine Biology FEB RAS, Far Eastern Federal University, Vladivostok, Russia
Expression of genes of pigment differentiation throughout the development and in cultured embryonic cells of the sand dollar *Scaphechinus mirabilis*
- 18 **Georgy Nevinsky**, S.E. Soboleva, N.I. Menzorova, P.S. Dmitrenok
SB RAS Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia
Enzymes, proteins, and soluble multi-protein complex of eggs of sea urchin *Strongylocentrotus intermedius*
- 19 **Rita Bernhardt**
Saarland University, Germany
Steroid hormone biosynthesis meets biotechnology
- 20 **Oleg Chernikov**, Hsiao-Wen Chiu, Lan-Hui Li, V.I. Molchanova, I.V. Chikalovets, Kuo-Feng Hua
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Polysaccharide from *Pseudoptero-gorgia americana* modulates immune response in macrophages
- 21 **Valentina Bashkatova**
P.K. Anokhin Research Institute of Normal Physiology, Moscow, Russia
Mechanisms of prenatal effects of caffeine in rats: role of nitric oxide
- 22 **Vladimir Gorbach**, V.N. Davydova, V.P. Glazunov, I.M. Yermak
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Liposomes as carrier for echinochrome
- 23 **Alexander Artvukov**, E.P. Kozlovskaya, L.N. Bogdanovich, N.M. Lupach, S.P. Kryzhanovskii, V.A. Stonik
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
Application of natural polyhydroxynaphthoquinone echinochrome A for treatment and prevention of atherosclerosis
- 24 **Andrey Hmelkov**, T.N. Zvyagintseva, N.M. Shevchenko, A.B. Rasin, S.P. Ermakova
G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC), Vladivostok, Russia
New fucoidan fractions from brown alga *Fucus evanescens*: structure and biological activity
- 25 **Valentina Kratasyuk**, Elena Esimbekova
Institute of Biophysics SB RAS, Federal Research Center "Krasnoyarsk Science Center SB RAS", Krasnoyarsk, Russia
Design in biotechnology: Can living organisms in bioassays be replaced on enzymes?

- 26 **Anna Grinkova**, N.E. Ustyuzhanina, N.E. Nifantiev
N.D. Zelinsky Institute of Organic Chemistry RAS, Moscow, Russia
Synthesis of hyaluronic acid related oligosaccharides

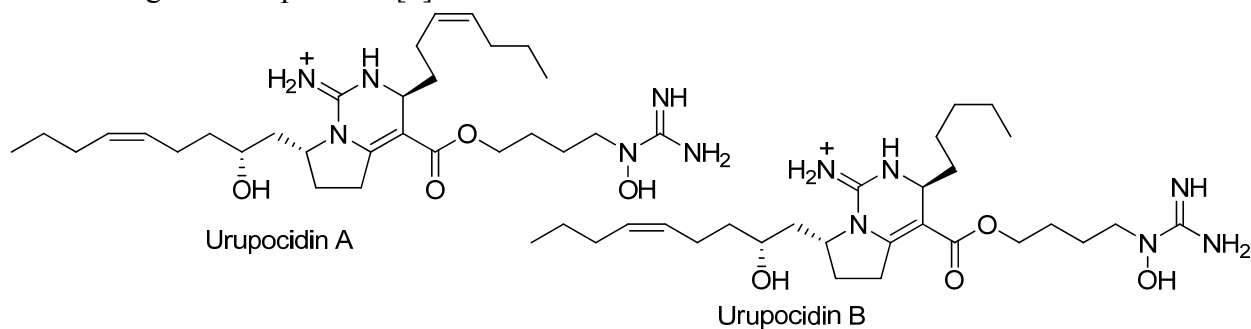
Oral presentation

SEARCH AND STRUCTURAL STUDIES OF SECONDARY METABOLITES FROM FAR EASTERN MARINE INVERTEBRATES

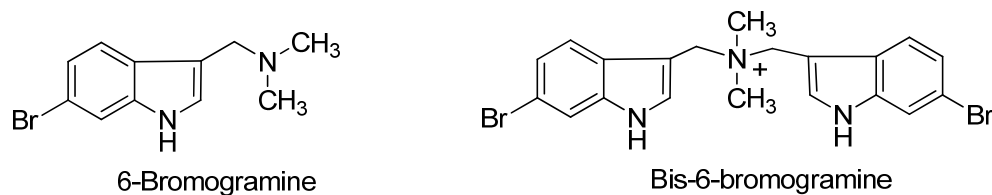
T.N. Makarieva, L.K. Shubina, A.G. Guzii, E.K. Kudryashova, and V.A. Stonik

G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia

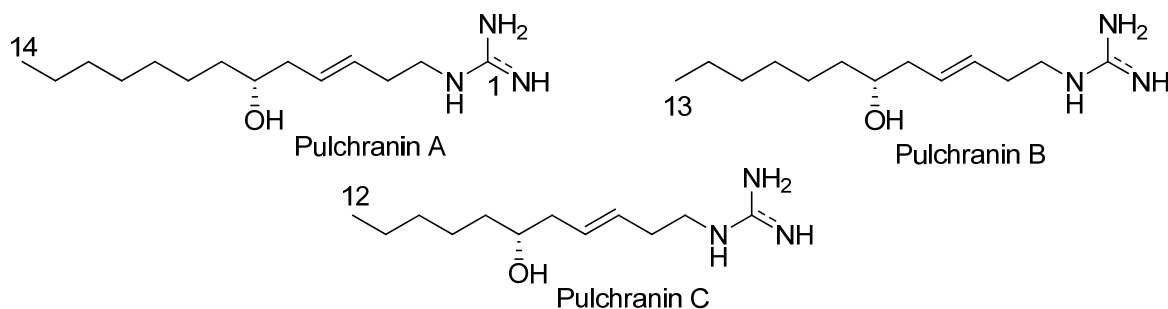
Marine invertebrates have long been known as a prolific source for the discovery of bioactive secondary metabolites. Most research in this area has been focused on invertebrates from tropical waters, but more recently the focus has shifted to the less accessible invertebrates of temperate and colder waters. A potentially fruitful opportunity for search of new marine secondary metabolites lies within the Northwestern Pacific region, where promising search for new natural products has been completed. The most common marine invertebrates of the region are sponges and echinoderms. In our laboratory about 300 natural compounds from numerous species of the Far Eastern marine invertebrates have been isolated, the corresponding chemical structures established, and biological activities studied. These substances belong to a wide variety of biogenetic classes including polar steroids (more 100), triterpene glycosides (about 100), and alkaloids (about 50). Up to day we have collected 3939 samples of marine organisms during 15 scientific cruises on board the research vessel “Akademik Oparin” at the period 1986 to 2017 from the Northwestern Pacific region mainly near the Kuril Islands. In result, urupocidins A and B with an unprecedented skeleton system have been isolated from the Far-Eastern sponge *Monanchora pulchra*, are noticeable examples of recently discovered novel marine bioactive secondary metabolites. Urupocidin A increases nitric oxide production in murine macrophages *via* inducing iNOS expression [1].



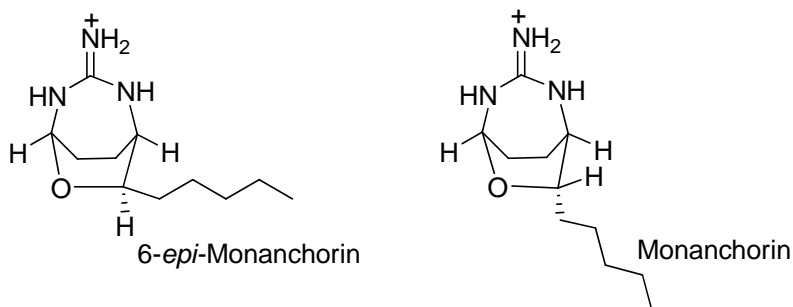
Two new natural products, 6-bromogramine and bis-6-bromogramine, inducing NF- κ B activity, have been isolated from the marine Far-Eastern hydroid *Abietinaria abietina* [2].



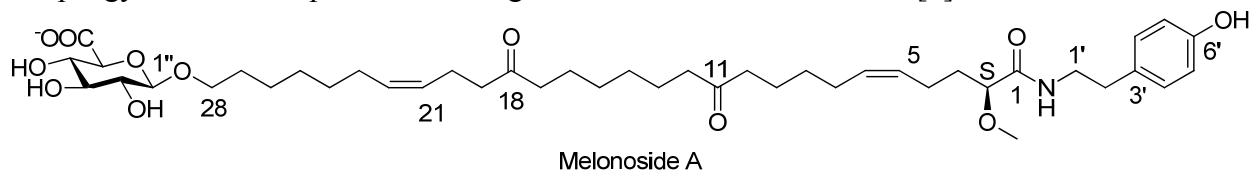
Pulchranin A, the first nonpeptide TRPV1 channel inhibitor, and two its homologous acyclic alkaloids were isolated from the Far Eastern marine sponge *Monanchora pulchra*. We have found that activity of pulchranins decreased in the row pulchranin A - pulchranin B - pulchranin C and in the row TRPV1- TRPV3 - TRPA1. So, we propose that the hydroxylated alkenyl moiety plays an important role in inhibitory activity of pulchranins [3-5].



As a result of the first study on secondary metabolites from the cosmopolitan bioluminescent marine tube polychaete *Chaetopterus variopedatus*, a new bicyclic guanidine alkaloid 6-*epi*-monanchorin along with the previously known monanchorin were isolated [6].



Unprecedented bipolar glycolipids, melonoside A and its analogues, have been isolated from the Far-Eastern marine sponge *Melonanchora kobjakovae*. Melonoside A induces autophagy of human cisplatin-resistant germinal tumor cells NCCIT-R [7].



Recently we have been isolated from Far Eastern sponge *Haliclona* sp. new structurally unique sulfated polyhydroxysteroids named by us gracilosulfates A-C. The structures of new secondary metabolites were elucidated by NMR, MS analysis and chemical transformations. Herein we discuss the diversity of unusual secondary metabolites from Far-Eastern marine invertebrates as well as the details of their structure elucidation and provide insights into their biological activities.

This work was supported by the Russian Science Foundation (Grant 17-14-01065).

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*Oral Presentation***METABOLOMIC APPROACHES IN THE STUDIES OF HOLOTHURIAN AND STARFISH GLYCOSIDES****P.S. Dmitrenok***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Metabolomics is a rapidly developing field of research based on the study of low molecular weight metabolic profiles of various objects - tissues, fluids, organs and etc. including whole organisms. Since both genetic factors and environmental factors affect the level of metabolites, the metabolic approach provides an assessment of the physiological state of the organism and helps identify potential chemical markers that can be used as indicators of physiological and pathological biological processes or their responses to changes in various factors. At the moment, metabolomics is successfully used in biology, medicine, toxicology, ecology, as well as in studies of the influence of environmental factors [1-3].

Metabolic fingerprinting and metabolic profiling are the main methods of metabolomic approaches [4]. The first is the analysis of biological objects on the basis of the common types of chromatograms formed by the qualitative and quantitative ratio of the including metabolites. This approach is aimed at a comparative analysis of the type of chromatograms that reflect changes in the disease or external effects on the body and covers the widest possible range of metabolites. Another type of metabolomic analysis - metabolic profiling – is an analysis in which a group of compounds of a certain class is studied. Metabolic profiling is used in the analysis of metabolites, belong to the same chemical class of substances or a particular biochemical pathway, it is carried out in order to establish or refine the biosynthetic pathways search biomarkers diseases, research target groups of metabolites, diagnosis or targets for drug research effects. Metabolic profiling is widely used to study the influence of environmental factors on organisms, for example, to study the change in the composition of secondary metabolites of plants [5].

Mass spectrometry has been playing an important role in the structural analysis of complex mixtures of natural products mainly due to its high sensitivity, rapid analysis time and selectivity. Liquid chromatography sequentially combined with mass spectrometry, more often using LS-ESI-MS methods, have been extensively applied to the analysis of complex mixtures of many natural compounds, and proved to be the appropriate analytical tool to obtain the necessary structural information for a great diversity of natural products. Metabolic profiling, including the analysis of low-molecular secondary metabolites in biological objects by modern HPLC-MS methods, has been widely used in recent years to study the metabolites of terrestrial plants and animals, for example, glycoside conjugates of phenolic substances, plant saponins, lipids and other compounds. However, the number of papers devoted to metabolic research of marine invertebrates is very limited.

We have applied this method for investigation of metabolite profiles of triterpene glycosides and polar steroid constituents of some Far Eastern holothurian and starfish, respectively. Marine glycosides and related highly polar compounds are a large group of secondary metabolites found in various marine organisms, in particular, in marine invertebrates.

Of particular interest are triterpene glycosides of holothurians and steroidal glycosides and related polar steroids of starfish. These substances have an original chemical structure,

significantly differing from similar metabolites of terrestrial origin, and exhibit a variety of physiological activities, including antitumor, antiviral, anti-inflammatory, analgesic, hemolytic, immunomodulating and other properties. Usually these substances are studied by isolation of individual compounds, and only in recent years there have been works devoted to the study of mixtures of triterpene glycosides of holothurians and steroid glycosides and related starfish compounds without the isolation of their individual components.

We have studied detailed mass spectrometric information about a wide range of individual previously known and new triterpene glycosides of holothurians and steroidal glycosides and related polar steroids of starfish with the aim of compiling databases of their MS/MS spectra. In particular, the features of mass spectrometric fragmentation of series of asterosaponins previously isolated from the starfishes *Lethasterias fusca* and *Aphelasterias japonica*, and 38 triterpene glycosides isolated earlier from the sea cucumber *Eupentacta fraudatrix* were obtained. We have also been studied mass spectrometry characteristics of five representatives of a rare type of steroid oligoglycosides starfish cyclic steroid glycosides that have trisaccharide chain cyclized between C-3 and C-6 of the aglycone isolated early from the starfish *Echinaster luzonicus*.

Mass spectra, including high resolution (HR) and tandem (MS/MS) ESI mass spectra, of several new sea cucumbers triterpene glycosides and starfish asterosaponins were also obtained. Thus, the new asterosaponin of aphelasteroside F isolated from the Far Eastern starfish *Aphelasterias japonica*, which has a new type of carbohydrate chain not previously encountered in starfish asterosaponins, 11 asterosaponins from the starfish of *Pentacaster regulus*, including seven new pentaregulosides A-G, two of which are representatives of the furostanoic steroid glycosides rare for starfish, and two new triterpene glycosides of fallaxosides B₁ and D₃ isolated from the Far Eastern holothurian *Cucumaria fallax*, having disulfated and trisulfated carbohydrate chains and unique aglycons, were investigated. The data obtained expand the mass spectrometric database that we are creating for carbohydrate-containing metabolites of echinoderms.

Metabolic profiling of triterpene glycosides of the sea cucumber *Eupentacta fraudatrix* widely distributed near the coast of Primorsky Krai was made. Metabolomic approach using LC-ESI-MS technique made it possible to identify known compounds and establish elemental composition and suggested the structures of new triterpene glycosides. LC-MS approach allowed a multitude of new as well as previously isolated triterpene glycosides to be characterized. Analyzed profile revealed at least 54 compounds, including 26 sulfated, 18 non-sulfated and 10 disulfated glycosides (Figure 1).

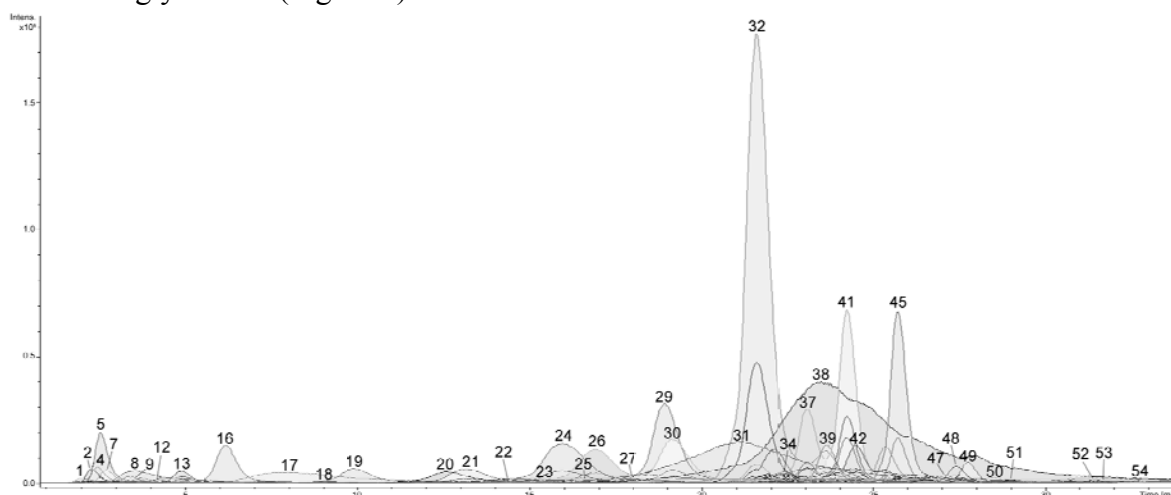


Figure 1. LC-ESI MS total compounds chromatogram of detected triterpene glycosides in negative ion mode (sulfated, disulfated and non-sulfated glycosides were detected as $[M - Na]^-$, $[M - 2Na]^{2-}$ and $[M - H]^-$ ions) in ethanol extract of sea cucumber *Eupentacta fraudatrix*.

In accordance with the proposed structures of oligosaccharide chains, all the detected triterpene glycosides of *E. fraudatrix* were subdivided into eleven groups (I-XI). Five of the eleven types of oligosaccharide chains were not found previously in *E. fraudatrix*. In addition, we discovered new glycosides having methylated glucose as the terminal monosaccharide residue.

Also the content of the detected triterpene glycosides in separate organs of the sea cucumber *E. fraudatrix* was studied by metabolic approach. The walls of the body contained the largest amounts of most of the detected triterpene glycosides, which confirms the supposed protective role of these substances. Differences in relative amounts of some compounds in different organs of this holothurian indicate additional biological functions of triterpene glycosides in the producing organism that need to be investigated.

Obtained data allowed us to propose a biosynthetic pathway for oligosaccharide chains in *E. fraudatrix* (Figure 2). These data correspond to theoretical biosynthetic pathway of oligosaccharide chains proposed early [6].

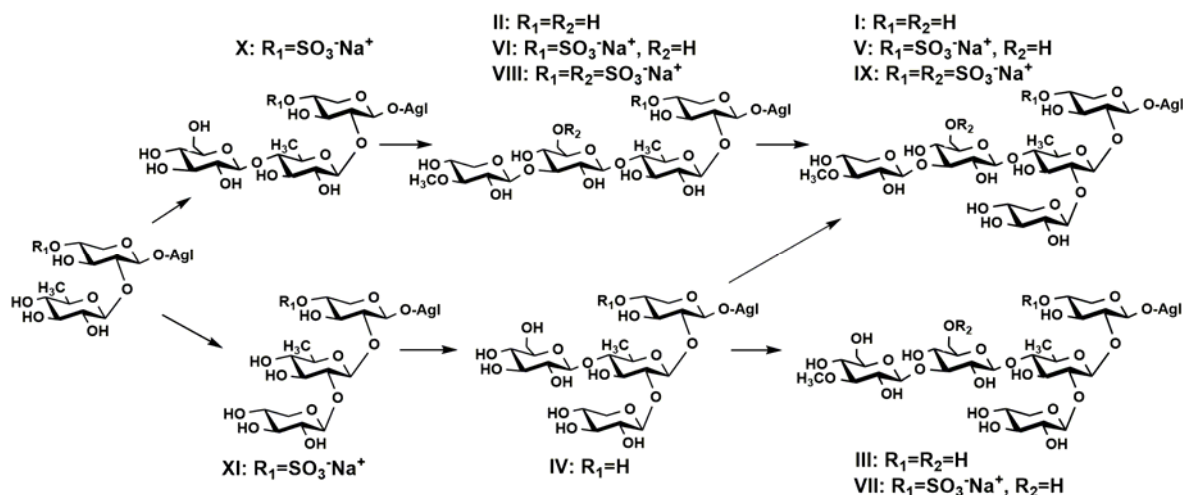


Figure 2. Hypothetic scheme of biosynthesis of oligosaccharide chains in *E. fraudatrix*.

Using a metabolic approach, the total fraction of steroid compounds from the starfish *Lethasterias fusca* was studied and the study of fraction of triterpene glycosides of the sea cucumber of the genus *Psolus* was started.

Our investigation demonstrated that LC-ESI MS and LC-ESI MS/MS approach are quite applicable for the profiling of holothurian triterpene glycosides and starfish polar steroid compounds in such complex mixtures as holothurian and starfish extracts and useful for searching of new structures, comparing metabolomic profiles of different these marine animal species and populations for ecological, dietary and biosynthesis studies.

The work was supported by Russian Science Foundation (RSF) grant № 16-13-10185.

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Oral presentation

THE COMBINATORIAL LIBRARY OF ACTINOPORINS FROM THE SEA ANEMONE *HETERACTIS CRISPA*

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The actinoporin family is one of four most common toxin protein families isolated from sea anemones together with sea anemone sodium channel inhibitory toxin family, type I subfamily, sea anemone type 3 potassium channel toxin family, and venom Kunitz-type family, sea anemone type 2 potassium channel toxin subfamily. It has been shown that the actinoporins are produced as isoforms; each actinoporin is encoded by its own gene.

The pore-forming mechanism has been studied in detail, but some stages of the process are still being discussed. To clarify the role of amino acid residues during membrane binding, wild-type actinoporins, as well as recombinant and mutant ones that are produced in *Escherichia coli* have been used. In general, the process of pore formation by actinoporins involves its binding to a sphingomyelin of cytoplasmic membranes through the aromatic POC site, transition of a N-terminal α -helical region (1–25 aa) to the lipid-water interface, oligomerization of 3–4, 8, or 9 monomers within the membrane interface, and the insertion of the N-terminal region into membrane hydrophobic core resulted in the creation of the functionally active protein-lipid pore. Actinoporin conformational transformation from the soluble state to the membrane-binding one is a fundamental α -PFT property that is directed at the disruption of biological targets.

Due to pore-forming activity, actinoporins represent an important model for studying of protein-membrane interactions as well as tools to investigation of action on target organs and different cell cultures and to creation of actinoporin immunoconjugates with different ligands for selective killing of parasite and tumor cells. StnII from *Stichodactyla helianthus* encapsulated into liposome have been recently reported to function as an adjuvant inducing a robust specific CTL response. Moreover, earlier we demonstrated that RTX-A from *Heteractis crispa* exhibited an antitumor effect and suppressed IGF-induced tumor transformation of JB6P + Cl41 mouse epithelial cells. This effect was found to be due to the induction of p53-independent apoptosis and the inhibition of the activity of the oncogenic nuclear factors AP-1 and NF- κ B.

Several actinoporin isoforms with molecular weights of 18995.5 to 19398.7 Da exhibiting a high hemolytic activity were isolated from the tropical sea anemone *H. crispa* using a combination of liquid chromatography techniques. The sequences of the genes encoding

actinoporins were identified, and the amino acid sequences of the new polypeptides belonging to the actinoporin family were obtained. The actinoporins differ in their isoelectric points, the number and localization of charged amino acid residues at the functionally important N-terminal fragment of the molecule, as well as in the charge of a tetrapeptide (amino acid residues 74–77) involved in an electrostatic interaction with the cytoplasmic membrane. The phylogenetic analysis revealed that actinoporin clustering is consistent with the division of sea anemones into superfamilies and families. The functional analysis of six recombinant actinoporins demonstrated that *H. crispa* actinoporin grouping was consistent with the different hemolytic activity of their representatives. According to molecular modeling data, we assume that the direction of the N-terminal dipole moment tightly reflects the actinoporins' ability to possess hemolytic activity.

The obtained data expand knowledge on the structural and functional relationships of actinoporins and contribute to our understanding of the functioning mechanism of these molecules, which is the basis for the development of compounds with a high biomedical potential.

Keywords: sea anemones, actinoporins, hemolytic activity.

Oral presentation

REACTIONS OF POLYHYDROXYNAPHTHAZARINS AND THEIR METHYL ETHERS WITH AQUEOUS AMMONIA

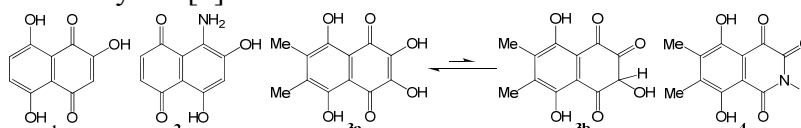
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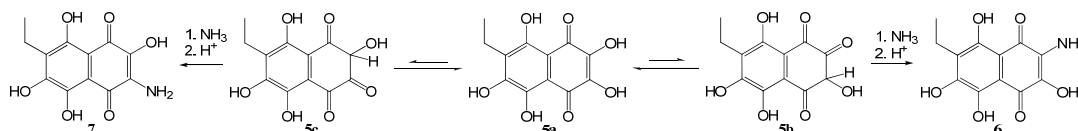
Naphthazarins (5,8-dihydroxy-1,4-naphthoquinones) bearing a different number of hydroxy groups in the β -positions of their skeletons are widely distributed in nature. In most cases, they are produced by marine invertebrates. All polyhydroxynaphthazarins are antioxidants, the effectiveness of which depends on the number of β -hydroxy groups and their positions [7]. Echinochrome A is the most famous among them. On the basis of this pigment a new drug «Histochrome» was created [5]. Recently, previously unknown metabolites, echinamines A and B, and spinamine have been isolated from different sea urchin species. These metabolites contain NH_2 groups in the β -positions of the cyclic backbone [6]. Theoretical studies of the mechanisms of antioxidant action of echinamines A and B showed that they are much more active as antioxidants than the parent echinochrome A [2]. In this connection, the development of methods for the amination of functionally substituted naphthazarins is of considerable interest. These approaches can open the way for the synthesis of both natural and related compounds containing amino functions in different positions of the naphthazarin skeleton.

We have shown that the reaction of 2-hydroxynaphthazarin (**1**) (naphthopurpurin) with a 25% aqueous solution of ammonia at room temperature proceeds rapidly (10 - 20 min), giving product **2** with a quantitative yield [4].

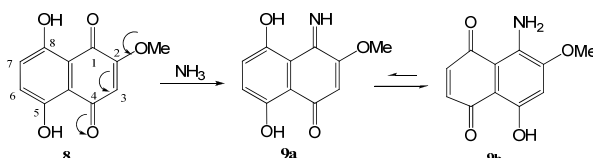


In the case of substrates with vicinal OH groups, the interaction with ammonia leads to the formation of products of a different type. Under amination reaction conditions 2,3-dihydroxynaphthazarin **3** are converted to 5,8-dihydroxyisoquinoline-1,3,4(2H)-trione derivatives **4** [1]. This can be explained by the fact that the substrate **3** exists partially in the tautomeric form **3b**. The reaction of electrophilic C-atom of $\text{C}(2)=\text{O}$ group of **3b** with NH_3 gives rise to amino intermediate that is turned to **4** by the action of O_2 .

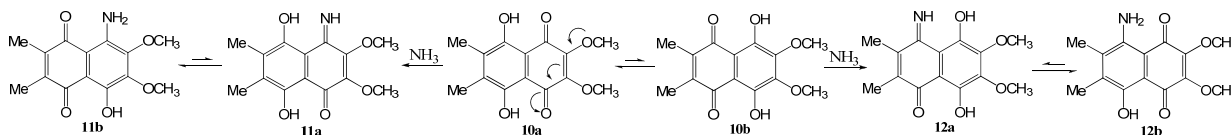
Substrates with three β -OH groups, for example, echinochrome A **5**, react with the formation of aminohydroxynaphthazarins **6** (48%) and **7** (47%) [4]. Because of the asymmetry of echinochrome A, the tautomeric forms **5b** and **5c** are not equivalent as a result of which the formation of isomers **6** and **7** is observed. In the case of substrate **5**, the reaction comes to rest at the stage of the formation of **6** and **7**. In the case of substrate **3**, the reaction proceeds via intermediates of the type **6** and **7** but is not brought to rest on this stage. We do not know yet why this happens. Undoubtedly, the nature of the C(6) and C(7) substituents at intermediates of these processes exerts a significant influence on the final result of these reactions. Substrate **3** has at these positions the relatively weak electron-donating substituents whereas substrate **5** has the ionized C(6) -OH group which is the strongest electron-donor.



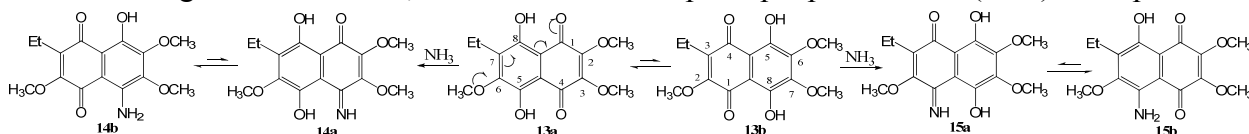
From the above, the question arises: will the aqueous solution of ammonia react with naphthazarin substrates where the β -OH groups are protected as OMe ethers? We found that the reaction of 2-methoxynaphthazarin **8** (naphthopurpurin monomethyl ether) with aqueous ammonia leads to the formation of aminonaphthoquinone **9** with a yield close to quantitative. In compound **8**, the most electrophilic center is the carbon atom of the C = O group at the position 1, since the electron-donating substituent at the position 2 partially deactivates the C = O group at the position 4.



Substrate **10** with vicinal OMe groups reacted with aqueous ammonia giving a mixture of products **11** (70%) and **12** (15%). As large as this difference in the yields of these products is a consequence of the strong dominance of tautomer **10a** in the tautomeric equilibrium of this substrate. This dominance is due to the electron-donating properties of OMe groups, that greatly exceed the same properties of Me groups. As a result, the electron density on the oxygen atoms of the C = O groups of tautomer **10a** will be larger than this density on the oxygen atoms of the C = O groups of tautomer **10b**. Such an electron density redistribution will increase the force of intramolecular hydrogen bond in tautomer **10a** compared to **10b** and will make **10a** dominate in the tautomeric mixture of the substrate **10**. Theoretical calculations have shown that the ratio of tautomers **10a** and **10b** in the alkaline medium is 70 to 30%.



As a result of the reaction of trimethyl ether of echinochrome **13** with aqueous ammonia, two products **14** (77%) and **15** (13%) were obtained. Their structures were assigned on the basis of the data of IR-, NMR-, and mass – spectra [3]. By analogy with what has been said above regarding the ratio of tautomers **10a** and **10b** in the case of substrate **10**, for substrate **13**, a tautomer **13a** with two OMe groups in the quinoid nucleus should dominate in the solution. Obviously, in the case of tautomer **13b** an attack of the nucleophile will proceed on the carbon atom of the C = O group at the position 1, that will result in the formation of a minor product **15b** (13%). In the case of tautomer **13a**, the direction of the nucleophile attack is not obvious as for tautomer **13b**. Probably, a definite orienting effect in this case is exerted by the C (6)-OMe group whose donor effect reduces the positive charge on the carbon atom of the C = O group at the position 1 and, as would be expected, makes an attack on the C = O group at the position 4 more advantageous. As a result, the formation of the principal product **14b** (77%) takes place.



As is evident from the foregoing, naphthazarins with one β -OH group at the position 2 react with NH_3 giving derivatives with the NH_2 group at the position 5(8); with two β -OH groups at the positions 2 and 3 give 2(3)-amino derivatives that are further oxidized to the corresponding

isoquinolinetriions by atmospheric O₂; with three β-OH groups at the positions 2, 3, and 6 give 2(3)-amino derivatives that are resistant to further oxidation with air oxygen. At the same time, OMe ethers of these naphthazarins react with NH₃ to form derivatives where the NH₂ group locates only at the position 5(8).

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Keywords: (poly)hydroxynaphthazarins, (poly)methoxynaphthazarins, amination, aminohydroxynaphthazarins, aminomethoxynaphthazarins.

*Oral presentation***SHIMADZU'S NEW CULTURE MEDIA ANALYZER PLATFORM FOR FUNDAMENTAL RESEARCH AND PROCESS DEVELOPMENT IN CELL CULTURES****Dmitry Farmakovskiy***Shimadzu Europa GmbH, Moscow, Russia*

The recent developed C2MAP™ system measures component changes in a culture supernatant as culturing progresses using LC/MS/MS. This system can be used for a wide range of applications, from basic research of cell cultures including pluripotent stem cells (iPS cells and ES cells), mesenchymal stem cells, and antibody-producing cells, to scaling up of culture volumes and actual process development.

Optimization and control of cell culture processes are essential to increase production efficiency of biopharmaceuticals. In the field of cell therapy, enhanced control of the culture process is also becoming important to reduce cell variability and improve consistency of mass production of the cells. Comprehensive monitoring of culture supernatant components gives researchers useful information for these purposes. However, current technologies for process monitoring are limited to measurement of pH, dissolving gases, and some small compounds such as glucose, glutamine, lactate, and ammonia.

Shimadzu Corporation has developed a “Cell Culture Media Analysis Platform, C2MAP system” that can provide simultaneous analysis methods for up to 95 components, making it suitable for component analysis to determine cell growth and status quality. The dedicated C2MAP TRENDS viewer software can graph component variations across multiple conditions for easier comparative analysis. The system can provide useful insights for the optimization of culture conditions in cell cultures by monitoring the consumption and depletion of media components during culturing, as well as the variation in metabolites secreted from cells.

The main features of the C2MAP System:

- ❖ Automated process from pretreatment to measurement for the culture supernatant analysis
 - With an automated process from pretreatment to measurement, automatic analysis can be performed at night and on non-working days.
 - The measurement workflow can be selected to match the actual culture.
 - Seamless analysis and management from pretreatment to LC/MS/MS measurement can be performed.
 - Pretreated samples are stocked on a microplate automatically to enable re-measurement with ease.

- ❖ Supports a wide range of Measurement compounds and culture supernatant samples
 - A total of 95 components, including major basal culture media components for animal cells and secreted metabolites, can be simultaneously analyzed at high speed.
 - Applicable to a wide range of cell culture media (iPS cells, ES cells, mesenchymal stem cells, T cells, and CHO cells).
 - Visualization of component variations in culture media
 - Temporal changes in the components can be displayed as trend graphs.
 - The results under multiple experimental conditions can be overlaid in the display, enabling comparative analysis.

For results obtained via LC/MS/MS, temporal changes in each component can be graphed by the dedicated viewer software. Analysts can monitor variations in metabolites secreted from cells and culture media components during cultivation, as well as display graphs of component comparisons with samples from different culture series. As a result, the consumption and depletion of culture media components, and changes in the amounts of metabolic components secreted from cells, can be observed, thereby providing useful insights into considerations of the optimal culture conditions and assessments of cellular status.

Oral presentation

SLOW-BINDING IRREVERSIBLE INHIBITORS OF RECOMBINANT ALPHA-GALACTOSIDASE FROM MARINE BACTERIA *PSEUDOALTEROMONAS* SP. KMM 701 AND ITS C494N MUTANT

I.Y. Bakunina, G.N. Likhatskaya, L.V. Slepchenko, L.A. Balabanova, L.K. Shubina, T.N. Makarieva

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O-glycoside hydrolases play an important role in the existence of micro- and macro-organisms. In bacteria, yeasts and fungi, these enzymes are involved in the degradation of various plant poly- and oligosaccharides that serve as a source of carbon and energy for growth of the organism, as well as performing various functions in organisms, including an offensive-defensive function. Modification or blocking of these functions by powerful selective inhibitors underlies the treatment of a number of infectious diseases, malignant tumors and genetic disorders. Inhibitors of enzymes are molecules that reduce or completely block the catalytic activity of an enzyme, so causing either complete death of cell or modification in the metabolic pathways.

The marine sponges are important sources of inhibitors of different class enzymes. We focus our attention to metabolites of marine sponge as inhibitors of glycosidases from marine bacteria. The effect of monanchomycaline B, monanhocicidin A and normonanhacidin A isolated from the Far Eastern sponge *Monanchora pulchra* on the activity of recombinant alpha-galactosidase from the marine bacterium *Pseudoalteromonas* sp. KMM 701 and to its mutant form C494N, as well as the recombinant alpha-N-acetylgalactosaminidase from the marine bacterium *Arenibacter latericius* KMM 426T was investigated. It was shown that all compounds are slow-binding irreversible inhibitors of alpha-galactosidase. Galactose that is a competitive inhibitor of alpha-galactosidase protects this enzyme from inactivation under the action of alkaloids. These compounds have no effect on the activity of alpha-N-acetylgalactosaminidase. The inactivation rate constants (k , min^{-1}) have been defined. The dependence of k value on the inhibitor concentrations has been established. It is shown that the inhibitory ability of alkaloids depends on their chemical structure, and the structural features of the active site of enzymes.

The theoretical model of 3D-structure of the alpha-galactosidase from the marine gammaproteobacterium *Pseudoalteromonas* sp. KMM 701 was constructed with the use of Homology module of MOE package on the basis of X-ray structure of *Lactobacillus acidophilus* alpha-galactosidase (PDB ID: 2XN2) as a template. The theoretical model of the structure of the

complexes of the alpha-galactosidase molecule with monanchomycaline B, monanhocicidin A and normonanhocicidin A were constructed with the method of molecular docking. Two binding sites for the alkaloids in the molecule of alpha-galactosidase are shown. Apart from the carboxyl groups of the catalytic residues Asp 451 and Asp516, the SH group of Cys494 in the active site of alpha-galactosidase takes part directly in the interaction with anchor parts of the molecules of guanidine alkaloids. A possible mechanism of interaction of monanchomycalin B with the active center of alpha-galactosidase is discussed, as well as biological role of the compounds in the life of sponge and symbionts. Detailed study of new marine inhibitors will provide the basis for future research.

The work was supported partly by FEB RAS grant № 18-4-026.

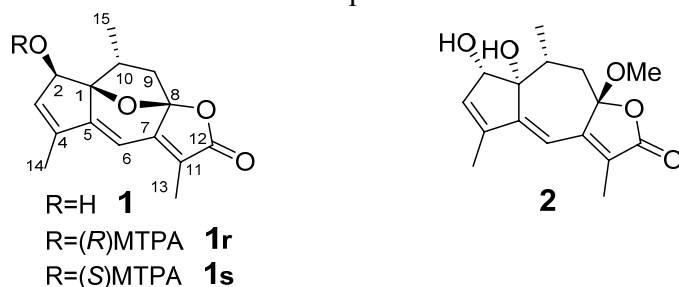
Oral Presentation

THE STUDIES ON STRUCTURES AND ABSOLUTE STEREOCHEMISTRY OF SECONDARY METABOLITES USING VARIOUS MODERN APPROACHES

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Determination of the absolute handedness, known as absolute configuration of chiral molecules, is an important step in any field of organic chemistry, especially for the structure elucidation of new natural products.



Two new unusual guaianesane sesquiterpenoids were isolated from the gorgonian *Menella woodin*. Their structures and relative configurations were determined by the extensive spectroscopic analysis. However, we were able to use a complex approach to determine the absolute configurations of the asymmetric centers of the compounds **1** and **2**. With the application of the Mosher's method, the *2R* configuration was determined. Then, the diverse array of computational techniques, including calculation of the coupling constants, ¹³C NMR chemical shifts, optical rotation and ECD allow to determine the absolute configurations of isolated compounds.

Quantum-chemical calculations, using B3LYP non-local exchange-correlation functional [1, 2] along with the 6-311G(d,p) basis set and polarisation-continuum model (PCM) [3], implemented using the Gaussian 03W package of programs [4], were used for further studies on the stereochemistry of **1** and to solve similar problems of **2**. In particular, the time-dependent density functional (TD-DFT) theory was used for the calculations of ECD spectra. The GIAO variant of theory was used for calculating NMR isotropic shielding constants.

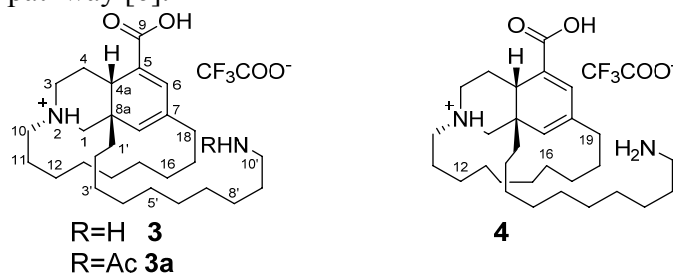
The comparison of statistically averaged theoretical ECD spectra of *1S,2R,8S,10R* (**1a**), *1S,2R,8S,10S* (**1b**) (all of them could demonstrate similar NOE correlations) with experimental ECD showed a good qualitative agreement between the experimental spectrum and calculated ECD of **1a** and **1b**, confirming the *1S,2R,8S* absolute stereochemistry in **1**, corresponding with NOE data and the results of Mosher's method application. However, the theoretical ECD spectra for **1a** and **1b** were quite similar doing impossible the C10 configuration determination. Thus, the limited capability of ECD spectroscopy, NMR data, and results of Mosher's method to complete the structure elucidation of **1** without additional information was brought out.

The comparison of theoretical and experimental chemical shifts δ_C of C9, C10 and δ_H of H9 α , H9 β as well as the comparison of calculated coupling constants $^2J_{H9\alpha, H9\beta}$, $^3J_{H9\alpha, H10}$ and $^3J_{H9\beta, H10}$ with corresponding experimental values allowed us to resolve this problem and establish the absolute stereochemistry of **1** as *1S,2R,8S,10R*. Calculated spin-spin coupling constants of **1a** were in agreement with the corresponding experimental values. All of this data assigned the signals of H9a at δ_H 1.68 as α , H9b at δ_H 2.68 as β and Me15 as α , respectively. In conclusion the specific optical rotation of *1S,2R,8S,10R-1* was calculated. The positive sign and statistically averaged $[\alpha]_{D,calc} = +514$ corresponded to the experimental value of $[\alpha]_{D,exp} = +217$. Thus, the structure of **1** was established on the basis of all discussed data as (*1S,2R,8S,10R*)-1,8-epoxy-2-hydroxy-guaian-3,5,7-trien-12,8-olide.

The strong negative specific optical rotation of **2** ($[\alpha]_D^{26} -206$) as well as antipodal ECD spectrum in comparison with **1** assumed a significant difference in the stereochemistry of the

compounds. The relative and absolute stereochemistry in **2** was determined on the basis of a comparison of ECD and NMR spectra, calculated for the energetically most preferable conformations of different stereoisomers of **2** with those obtained for **2** experimentally. Specifically, the comparison of experimental and calculated NMR data showed that only the $1R^*,2S^*,8S^*,10R^*$ structure with an equatorial position of Me15 correctly reproduces all features of the recorded NMR spectra. Attempts to assign the recorded NMR data to any other stereoisomer showed significant differences between observed and calculated constants and $\Delta\delta_{C(10-9)}$ values. The calculated ECD for the $(1R,2S,8S,10R)$ stereoisomer of **2** showed a similar CD curve with the experimental ECD spectrum. Finally, the specific optical rotation of $(1R,2S,8S,10R)$ -**2** was calculated. The negative sign and large value of statistically averaged $[\alpha]_{D,\text{calc}} = -594$ was in accordance with the experimental value $[\alpha]_{D,\text{exp}} = -206$ and proved the $1R,2S,8S,10R$ stereochemistry of **2** [5].

The quantum chemical modeling was also introduced for the absolute configurations elucidation in the lissodendoric acids A (**3**) and B (**4**), isolated from the sponge *Lissodendoryx florida*. The structures of the first representatives of a new group of manzamine-related alkaloids with previously unknown skeleton systems were determined by the extensive spectroscopic analysis together with chemical transformations. The comparison of statistically averaged theoretical ECD spectra of both enantiomers of acetylated lissodendoric acid A (**3a**) with experimental ECD showed a good agreement between the curves, confirming the $(4aS,8aS)$ absolute stereochemistry (Fig. 2), corresponding with NOE data and the possible biogenetic pathway [6].



The achieved results in the field of structural research of new natural products demonstrate the advantages of an integrated approach, including methods of quantum chemical modeling, directed chemical transformations together with consideration of biosynthesis pathway.

The work was supported by grant № 17-14-01065 from the RSF (Russian Science Foundation).

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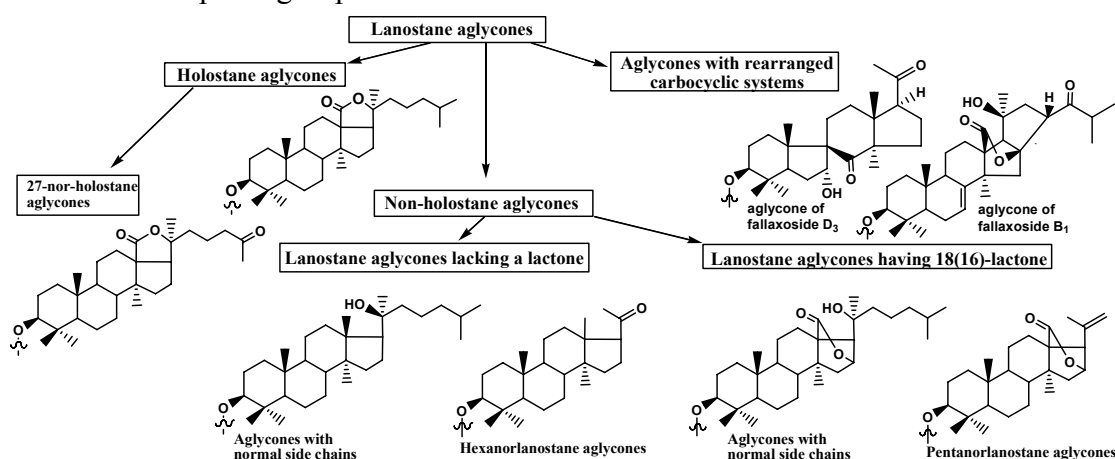
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STRUCTURAL DIVERSITY AND SOME BIOSYNTHETIC PECULIARITIES OF TRITERPENE GLYCOSIDES FROM THE SEA CUCUMBERS

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Sea cucumbers (class Holothuroidea) are the marine invertebrates belonging to the phylum Echinodermata biosynthesizing unusual for other animals metabolites – triterpene glycosides. The lanostane aglycones and carbohydrate chains comprise the molecules of triterpene glycosides. There are some structural types of the aglycones: holostane type (lanostane derivatives with 18(20)-lactone) and non-holostane type, which is subdivided into groups having 18(16)-lactone or lacking a lactone. There are also the aglycones with shortened or normal side chains within each group of the aglycones. Carbohydrate chains of these compounds differ from each other by the diverse monosaccharide residues composing them, by the number and sequence of the residues and therefore by the architecture. Some of the sugar chains contain from one to three sulphate groups.



The applying of modern techniques of isolation and structural elucidation allowed the discovering of some minor glycosides possessing very interesting structural features. Some glycosides are characterized by unusual aglycones [1 and references herein]. Among them are: synaptoside A₁ from *Synapta maculata* having 7-keto-8(9)-en fragment in holostane aglycone, cucumariosides A₈ and A₉ from *Eupentacta fraudatrix* having unique 18,20-dihydroxy fragment in the aglycones without lactone ring, cucumarioside A₁₂ from the same sea cucumber and 27-nor-25-oxo-holotoxin A₁ from *Psolus fabricii* the representatives of very rare 27-nor-triterpenoids; cucumarioside H₈ from *E. fraudatrix* and magnumoside A₁ from *Massinium magnum* having unique 16(22)- and 20(24)-epoxy-groups in the aglycones, correspondingly; unprecedented octanorlanostane aglycone of cladoloside C₄ from *Cladolabes schmeltzii*. Finally, the series of glycosides (fallaxosides) with uncommon aglycones was found in the sea cucumber *Cucumaria fallax*. Majority of them contained non-holostane aglycones without lactone having 8(9)-double bond and oxygen-containing functionalities at C-7 and C-11. Two compounds – fallaxosides B₁ and D₃ characterized by unprecedented rearranged lanostane carbocyclic systems in their aglycones. The first of the above has an 18(16)-lactone and additional 16,23-five-membered cycle; the latter has no a double bond, a lactone ring and a side chain and contain two cyclohexane and two cyclopentane rings and a spiro carbon.

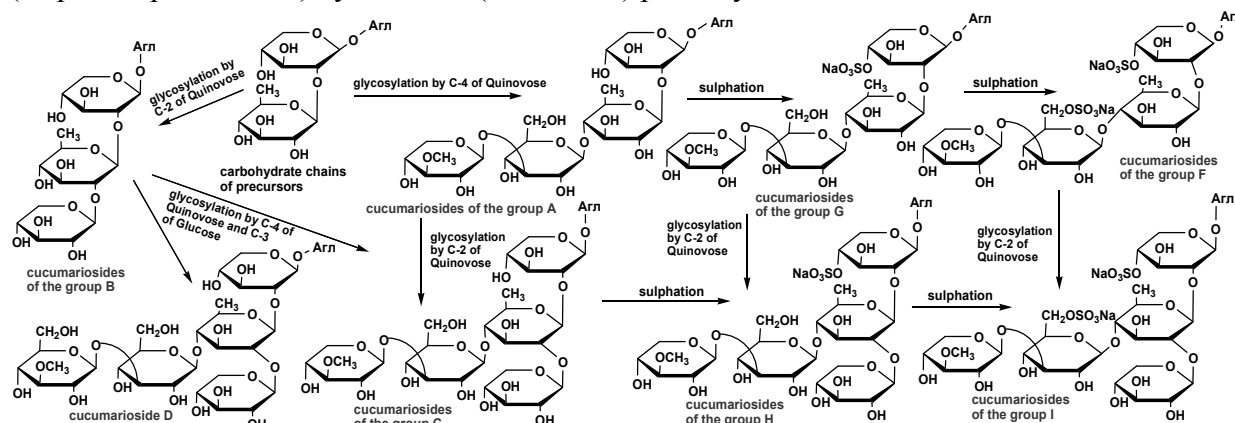
The pathways of biosynthetic transformations of these unique aglycones were suggested. Thus fallaxoside B₁ was formed as a result of an enzymatic intramolecular aldolic condensation of 1,6-diketo precursor leading to the formation of additional C-23–C-16 covalent bond. The formation of fallaxoside D₃ was explained by the proton induced Meinwald or pinacol-pinacolone-like rearrangement of 8,9-epoxy or 8,9-dihydroxy precursor, correspondingly, that leads to shifting of 7(8)-covalent bond to 7(9)-position with a size decreasing of cycle B to five-member one and formation of spiro-quaternary center between the rings B and C and a keto group at C-8 position [2].

Some uncommon structural features were also found in carbohydrate chains of the glycosides. The glycosides of *Synapta maculata* were characterized by pentasaccharide sulphated carbohydrate moiety, containing 3-O-methylglucuronic acid, which was found first in the glycosides of sea cucumbers. Moreover it does not occur in the glycosides from plants or sponges but comprises the capsule polysaccharides of bacteria. The sugar chains of the glycosides from *Staurocucumis turqueti* and *S. liouvillei* contain rare 3-O-methylquinovose residue that is considered as chemotaxonomic character of the genus *Staurocucumis*. Cucumariosides of the group B from *E. fraudatrix* and colochiroside E from *Colochirus robustus*, correspondingly, contain linear and branched trisaccharide chains. Cladoloside N from *Cladolabes schmeltzii* has three xylose residues (as first, second and third units) and cladolosides of the groups F and H have two quinovose residues (as second and fifth units) in the carbohydrate chains. High-polar glycosides with sulphated hexasaccharide sugar chains were first discovered in the same sea cucumber. Some of cladolosides were characterized by the presence of non-methylated sugars as terminal units in hexasaccharide chains. Colochiroside E from *C. robustus*, cladoloside J₁ from *C. schmeltzii*, psolusoside B from *Psolus fabricii* and kurilosides A and C from *Thyonidium kurilensis* were characterized by the unusual architecture of sugar chains.

So, having the data array concerning the structural diversity of triterpene glycosides from the sea cucumbers, we can analyze their biogenetic relationships and biosynthetic pathways. The species *Eupentacta fraudatrix* and *Cladolabes schmeltzii* are convenient objects for the analysis of carbohydrate chains biosynthesis since the high diversity of these parts of their glycosides. The synthesis and elongation of glycosides sugar moieties occur through the consequent attachment of monosaccharides to the certain positions of the forming chain. The sulphation is frequently considered as a final stage of sugar chain biosynthesis.

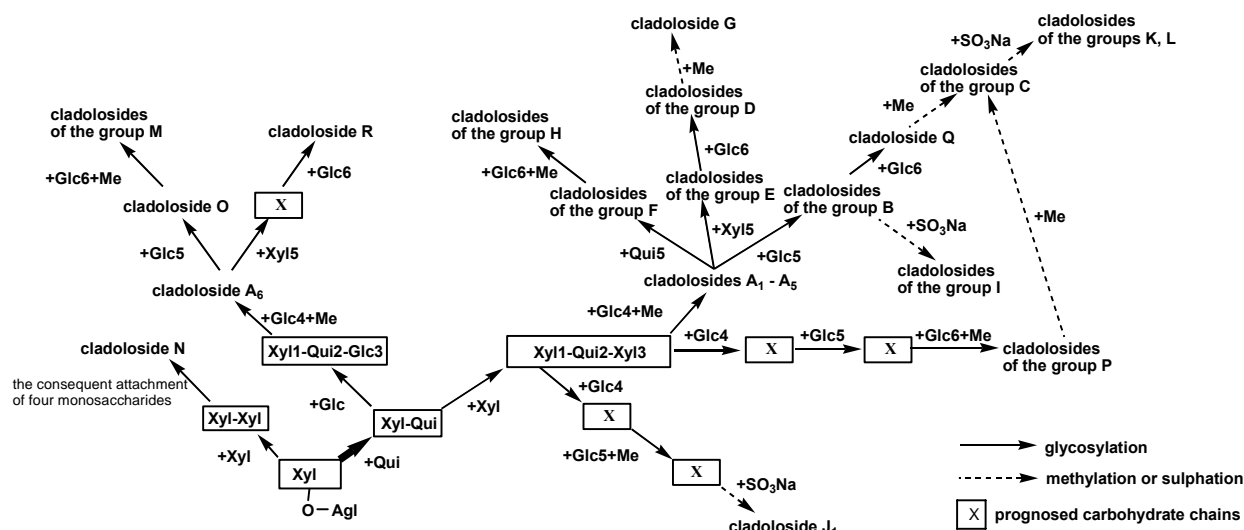
The glycosides of *E. fraudatrix* – cucumariosides of the groups A, B, C, D, F, G, H and I, contain trisaccharide, tetrasaccharide and pentasaccharide chains with or without sulphate groups [3 and references herein, 4]. Cucumariosides of the group A – linear nonsulphated tetraosides could be considered as “basic” group which is actively transformed in the process of biosynthesis. Its sulphation leads to the formation of the chains of cucumariosides belonging to the groups G and F and its subsequent glycosylation by C-2 of quinovose resulted in the formation of cucumariosides of the groups H and I. The formation of cucumariosides of the group C occurred by glycosylation of sugar chain of cucumariosides of the group A without prior sulphation. So, sulphation and glycosylation are the alternative processes leading to the synthesis of different groups of cucumariosides. The minor triosides – cucumariosides of the group B also could be the biosynthetic precursors of pentaosides belonging to the groups of cucumariosides C, D, H and I when the glycosylation by C-2 of quinovose precedes the glycosylation by C-4 of this residue. Thus the biosynthesis of glycosides carbohydrate chains in *E. fraudatrix* is a mosaic type as its different stages can be shifted in relation to each other in time (heterochrony) and

proceed in different consecutions. The result of such mosaicism is the formation of final products (sulphated pentaosides) by different (alternative) pathways.



The glycosides of sea cucumber *C. schmeltzii* are characterized by the extreme diversity (19 types) of carbohydrate chains. When analyzing their biogenesis it becomes obvious that each type of chain is biosynthesized by one pathway only as individual consequence of the glycosylation reactions. So this situation is opposite to that observed in *E. fraudatrix*. The first step of diversification of biosynthetic pathways of cladolosides occurs after the formation of xylosides of the aglycones. The mainstream of biosynthesis is the glycosylation of the first xylose residue by the quinovose leading to the formation of all the cladolosides with one exception (the attachment of another xylose unit occurs in the process of cladolide N formation). All consistencies of glycosylation reactions pass through the branching points. At the most branched point cladolosides of the group A with tetrasaccharide chains are located similarly to cucumariosides of the group A in *E. fraudatrix*.

The sulphation of cladolosides always occurs in final biosynthetic stages since sulphate group attaches to terminal methylated monosaccharide residues of penta- and hexasaccharide chains. The methylation of sugar chains is a “termination signal” stopping further elongation of a chain. The presence in some groups of cladolosides of non-methylated terminal residues indicates the attachment of O-methyl directly to synthesizing sugar chain, but not to the monosaccharide that consequently glycosylates forming chain. However in cladolosides 3-O-methylation is not always a final stage for the whole molecule because after the introduction of this termination group to one half-chain (upper or bottom) elongation of another half-chain could proceed. The time shifting of these stages relative to each other (heterochrony), characteristic for the mosaic type of biosynthesis, lead to the formation of carbohydrate chains with terminal residues with or without 3-O-methyl group in the fourth or sixth positions of the chains.



Thus, comparison of metabolic networks of carbohydrate chains of the glycosides from *E. fraudatrix* and *C. schmeltzii* showed different degree of mosaicism of their biosynthesis. Classical mosaic type of biosynthesis is inherent for the sugar moieties of *E. fraudatrix*. Whereas biosynthesis of carbohydrate parts of glycosides in *C. schmeltzii* has more regulatory (strictly directed) nature but with some character traits of mosaicism.

The work was supported by RFBR grant № 16-04-00010.

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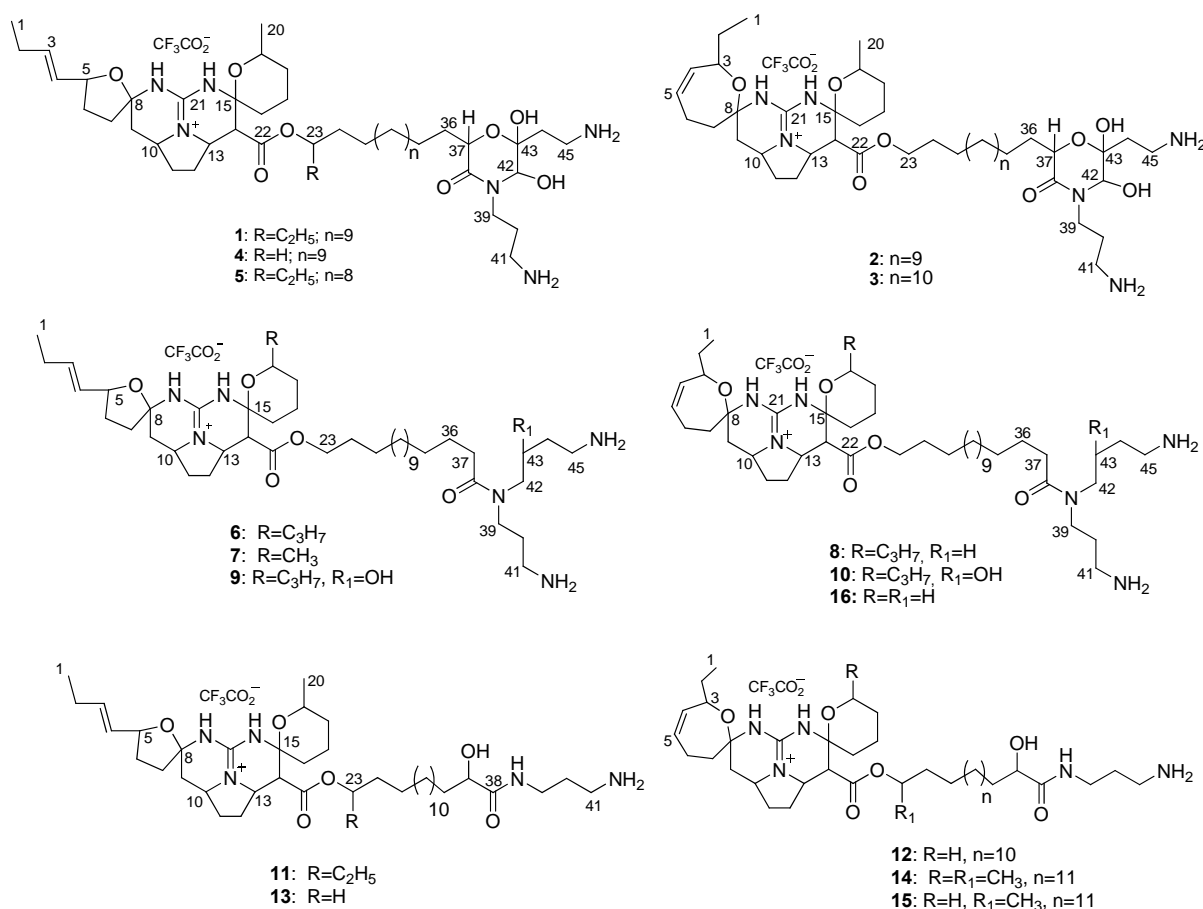
Oral Presentation

STRUCTURAL STUDIES OF PENTACYCLIC GUANIDINE ALKALOIDS FROM THE FAR EASTERN MARINE SPONGE *MONANCHORA PULCHRA*

K.M. Tabakmakher, T.N. Makarieva, A.G. Guzii, L.K. Shubina, S.A. Dyshlovoy, A.S. Kuzmich, V.A. Denisenko, P.S. Dmitrenok, R.S. Popov

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Pentacyclic guanidine alkaloids (PGA) are a unique group of secondary metabolites of marine sponges, interesting for its unusual chemical structure and a wide range of biological activity. Far Eastern sponge *Monanchora pulchra* is a rich source of these compounds. In the period from 2011 to 2017 we studied the alkaloid composition of 12 samples of this sponge, collected near the Kurile Islands. As a result, 16 PGAs were isolated. Complex use of methods such as NMR spectroscopy (^1H , ^{13}C , ^1H - ^1H -COSY, HSQC, HMBC, NOESY, ROESY, TOCSY), mass spectrometry (MALDI-TOF, ESI, ESI MS/MS) and chemical transformations has shown that 14 compounds are new representatives of the investigated class - monanchocidins B-E (**2-5**) [1], monanchomycalins A (**6**), B (**7**), C (**8**) [2, 3], monanchoxymycalins A (**9**), B (**10**) [5] and normonanchocidins A (**11**), B (**12**), D (**13**), G (**14**), H (**15**) [4, 6] and 2 are known earlier – monanchocidin A (**1**) [7] and ptylomycalin A (**16**) [8]. Some of the metabolites found contain fragments or a combination thereof that were not previously detected in PGA.



Based on the obtained new structural information and literature data, a scheme of possible biosynthetic pathways for the formation of these secondary metabolites was developed

and the following conclusions were made: PGA precursors (fatty acid derivatives) can differ not only in length of the carbon skeleton, but also in position and in the number of hydroxyl groups; PGA with a cycle of morpholinone in the "anchor" part can be biosynthesized from precursors containing a block of spermidine or oxyspermidine; in the formation of a lipid bridge between the "anchor" and "vessel" parts of the molecule, may be involved derivatives of not only ω - and ω -3-, but also ω -2-hydroxy fatty acids

For the isolated substances, the biological effect on the line of human tumor cells HL-60, THP-1, MDA-MB-231, HeLa and normal mouse epithelial cells of the JB6 P + Cl41 line was studied. It was shown that the studied alkaloids show high antitumor cytotoxic activity, as well as cancer-preventive activity in non-toxic concentrations. Partially studied mechanisms of antitumor activity of these alkaloids. In addition, some new metabolites in noncytotoxic concentrations significantly inhibit the ability to migrate HeLa cell line. Thus, these substances can be promising as a means of preventing the formation of tumor cells and their spread in the body.

The work was supported by RSF grant № 17-14-01065.

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*Oral Presentation***BACTERIAL CYTOCHROMES P450 ARE HIGHLY EFFICIENT AND PROMISING TERPENE HYDROXYLASES****Rita Bernhardt**

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Cytochromes P450 are the first enzymes to modify terpene synthase products. Although major advances concerning the engineering of plant P450s have been made, these enzymes still pose great challenges, especially in terms of microbial expression. Therefore, soluble bacterial P450s catalyzing terpene hydroxylation came into the focus of interest for biotechnological production of terpenoid compounds thus demonstrating the potential of these P450 enzymes for the production of terpenoids for the fragrance, flavor and pharmaceutical industry.

We have demonstrated highly selective hydroxylations of di- and triterpenes by members of the CYP106 family of *Bacillus megaterium*, as well as by cytochromes P450 from *Sorangium cellulosum* Soce56. The 3D structure of several of these P450s has been resolved and the structural basis for highly selective substrate hydroxylation has been explained. Moreover, highly selective hydroxylations of α - and β -ionone as well as nootkatone and other terpenes were performed using different members of myxobacterial P450s and a valuable toolbox for the production of terpenoids has been created.

Taken together, bacterial terpene synthases and P450s are promising tools for the production of modified terpenes.

MOLECULAR CLONING OF SEA CUCUMBER OXIDOSQUALENE CYCLASES

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Sea cucumbers contain rare Δ^7 or $\Delta^{9(11)}$ -sterols including methyl sterols in their cell membranes instead of main zoosterol – cholesterol. Also, sea cucumbers produce a huge number of diverse triterpene glycosides with 7(8)- or 9(11)-double bond in their aglycones (Kalinovskaya N.I., et al., 1984, Stonik et al., 1998., Kalinin et al., 2005). It has been suggested that the holothuroid sterols play an important role in resistance of the cell membrane to a lytic action of saponins due to their ineffective interaction with each other compared to cholesterol-saponin one (Makarieva et al., 1993).

Biosynthesis of sea cucumber sterols and saponins is still a mystery taking into account the unusual cyclization of oxidosqualene into parkeol, the presence of unusual 4,14-dimethyl- and 14-methyl- $\Delta^9(11)$ -sterols, and the inability of polycyclic precursors (parkeol, lanosterol and cycloartenol) to be transformed into glycosides. In addition, it is still difficult to explain how glycosides with a 7(8)-double bond acquire 9β -H configuration (Silchenko et al., 2012). Previously, Makarieva et al. (1993) proposed the existence of two pathways of sterol biosynthesis in sea cucumber *Eupentacta fraudatrix*. The first pathway involves de novo biosynthesis of 14-methylcholest-9(11)-en-3 β -ol from parkeol and the second one involves biosynthesis of triterpene saponins from lanosterol. Later, it was suggested that some glycosides with 7(8)-double bond can be biosynthesized via cyclization of oxidosqualene into 9β -H-lanosta-7,24-dien-3 β -ol (Silchenko et al., 2012). Therefore, it can be assumed that an oxidosqualene cyclase from sea cucumbers must have structural features in an active center compared with those of other animals. Herein we report the results of molecular identification of OSC genes from the holothurians *E. fraudatrix* as well as structure and evolutionary analysis of these enzymes.

Two partial-length cDNAs, encoding OSC1 and OSC2, were obtained by reverse transcription, RACE and molecular cloning from the total RNA isolated from body wall and intestine samples of the sea cucumbers *E. fraudatrix*. Sequence comparison showed that OSC1 and OSC2 are 89% identical to each other and share 51–62% identity to the other animal OSCs. Structure comparison analysis identified the conserved SQCY_1 and ISOPREN_C2 domains characteristic for class II terpene synthases. Both sequences showed the presence conservative motifs, which are important for carbocation formation and protein structure maintenance. However, OSC1 and OSC2 have some substitutions of functionally important residues in the active center that could be responsible for the formation 7(8)- or 9(11)-double bond in a triterpene nucleus. Phylogenetic tree of OSCs demonstrates that OSCs of *E. fraudatrix* do not cluster with other echinoderm OSCs but takes a position basal to the clade of other animal OSCs (100% bootstrap support).

Thus, the coevolution of sea cucumber saponin-sterol pairs is triggered by the appearance of the second and extensive diversification of both OSC genes.

The work was supported by the RSCF grant № 17-14-01065.

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*Oral Presentation***MARINE BACTERIAL ENZYMES FOR MOLECULAR GENETICS AND STRUCTURE-FUNCTION STUDIES**

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The marine environment conditions involving low temperature, high pressure and high salinity made the marine habitants to synthesize such enzymes, the characteristic features of which were the higher catalytic effectiveness and unusual specificity compared to the terrestrial counterparts due to their molecular flexibility and still poor explored biological function. The recombinant alkaline phosphatase from the marine bacterium *Cobetia amphilecti* KMM 269 (CmAP) with the higher activity ($\geq 12,000$ U/mg) among the known analogues was successfully applied for genetically modifying the lectins from marine invertebrates as well as the porin of pathogen *Yersinia pseudotuberculosis*, which are promise diagnostic agents in the cancer and infectious diseases, respectively. The CmAP activity of the recombinant bifunctional GalNAc/Gal-specific lectin (CGL) from the mussel *Crenomytilus grayanus* well extrapolated the results of substitution of each amino acid residues affected the mucin-binding activity on *in silico* analysis of binding mechanisms of CGL to galactose, globotriose and porcine stomach mucin. It has been found for the first time that the CGL binding effectiveness depends on the monosaccharide composition of oligosaccharide due to the ability to form additional hydrogen bonds with both the terminal galactose and neighboring residues. The CmAP module was used to investigate the specificity of CGL toward tumor markers CA19-9, CA125, CA72-4, carcinoembryonic antigens (CEA), alpha-fetoprotein (AFP) and prostate-specific antigen (PSA). CGL genetically fused with CmAP was shown to be promise tool for the development of enzyme-linked lectin assay for identify tumor markers CEA and CA19-9 in clinical specimens. The use of alkaline phosphatase CmAP in the genetically labelled CGL and OmpF-porin hybrid proteins for the diagnostics eliminated the use of enzyme-labeled secondary antibodies necessary for detecting specific antibodies in the biological samples to simplify the procedure and shorten the analysis time. The CmAP/OmpF-porin functionality was confirmed by the binding of

antibodies to OmpF-porin in murine antisera, as well as in the sera of patients with pseudotuberculosis. Moreover, the CmAP activity was stimulated in the presence of specific antibodies to OmpF porin, improving the diagnostic relevance.

Apart from the structure-function studies, the marine bacterial enzymes could be used for the elucidation of some cell properties such as their growth or biofilm formation at the molecular genetic level. CmAP inhibited the growth of the MDA-MB-231 cell line (breast adenocarcinoma) by 44.9% at concentration 2.3 U/mg after 48h of their enzymatic treatment and incubation. The dose-dependent effect of CmAP was observed on both the mature and *de novo* biofilms of most known pathogens. Remarkably, the α -galactosidase from the marine bacterium *Pseudoalteromonas* sp. KMM 701 also showed an effect on the level of biofilm-regulating genes expression in the widespread pathogen *Pseudomonas aeruginosa*. However, the question of which the molecular mechanisms trigger these cell processes has yet to be investigated in detail.

The work was supported by the program “Far East” grant № 18-4-05.

*Oral Presentation***GENOMIC APPROACH TO THE SEARCH FOR ENZYMES FOR TRITERPENE GLYCOSIDE TRANSFORMATION****E.P. Bystritskaya, N.U. Chernysheva, M.P. Isaeva***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Triterpene glycosides, known as holothurins (or saponins), are the main bioactive metabolites of sea cucumbers (class Holothuroidea) with a wide range of biological activities that allows to consider them as very promising anticancer, antifungal, antiprotozoal and antiviral therapeutic agents [1]. Many holothurins are sulfated or acetylated triterpene glycosides having two parts: aglycone and glycone. Cytotoxic activity could be affected by the side chain position and number of monosaccharide units and sulfate groups [2].

Microbial transformation of holothurins has provided novel low toxicity derivatives with an enhanced bioactivity that are potentially useful for pharmacological studies. Based on the structure of triterpene glycosides, the following classes of bacterial enzymes are of interest for bioconversion: glycoside hydrolases and glycosyltransferases, sulfatases and sulfotransferases, monooxygenases and oxidoreductases.

The genomes of representative marine Flavobacteriaceae (*Mesonia*, *Vitellibacter*, *Zobellia*, and *Formosa*) have been analyzed to understand the occurrence and diversity of genes involved in bioconversion/degradation of glycosides. The family members reveal a huge potential for degradation of (poly)saccharides including sulfated ones. We performed *in silico* search for genes encoding members of GH1, 3, 4, 109, GT1, 4, and GMC oxidoreductase families. The genome survey showed that glycoside hydrolases, particularly beta-glucosidases of GH1 and GH3, and GMC oxidoreductases from *Zobellia*, and *Formosa* might be good candidates to hydrolyze carbohydrate moieties from triterpene glycosides. The gene sequences were subjected to an analysis of their gene duplication/reduction and evolutionary relationships. Thus, genome analysis revealed a huge potential for glycoside degradation/bioconversion.

The work was supported by the RSCF grant № 17-14-01065.

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SEAGRASS-DERIVED FUNGI AS A SOURCE OF BIOACTIVE COMPOUNDS

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Marine-derived fungi are a prolific source of new secondary metabolites many of which are biologically active [1-3]. As part of our ongoing search for structurally novel and bioactive metabolites we have isolated 17 new compounds from two fungi *Penicillium thomii*, associated with seagrass *Zostera marina* (Troitsa bay, Sea of Japan), including five new eudesmane-type sesquiterpenes – thomimarines A-E (**1-5**) [4, 5] (Figure 1) from the *P. thomii* KMM 4667 and 12 new polyketides with “decalin” moiety – zosteropenillines A-L (**6-17**) (Figure 2) from the *P. thomii* KMM 4674 [6] (Figure 1). Their structures were established based on spectroscopic methods. The absolute configurations of thomimarines A-E (**1-5**) and zosteropenillines B-D (**7-9**) were determined by time-dependent density functional theory (TD-DFT) calculations of ECD spectra. The absolute configuration of zosteropenilline A (**6**) was determined by a combination of the modified Mosher’s method, X-ray analysis and NOESY data (Figure 3).

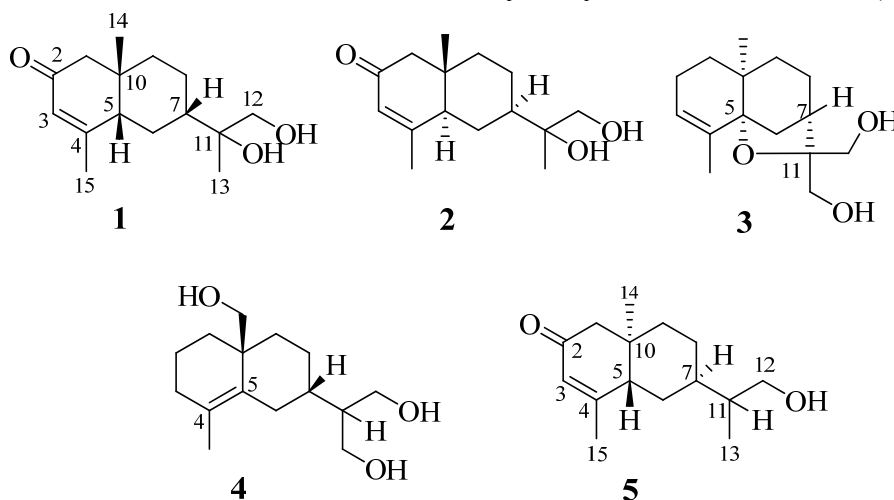


Figure 1. Chemical structures of thomimarines A-E (**1-5**)

It was found that compounds **1**, **4**, **5**, **6**, **13** and **15** at concentration of 10.0 μ M induced a moderate down-regulation of NO production in macrophages stimulated with LPS. NO level in these cells was decreased by 24.9% \pm 0.9, 20.9% \pm 5.7, 22.5 \pm 5.1%, 27.7% \pm 1.8, 20.6% \pm 1.2 and 22.3% \pm 3.8, respectively, compare to control cells pretreated with LPS. The most effective substance was compound **2** exhibited the maximal pronounced inhibition of NO formation in LPS-stimulated RAW 264.7 cells by 43.4% \pm 1.5. The effect of zosteropenillines **6-8**, **12**, **13**, **15** and **16** on the viability of human drug-resistant prostate cancer cells PC3 as well as on autophagy in these cancer cells was examined. The results suggest that the investigated compounds are able to inhibit autophagy at non-cytotoxic concentrations and may sensitize human cancer cells to cytotoxic anticancer drugs.

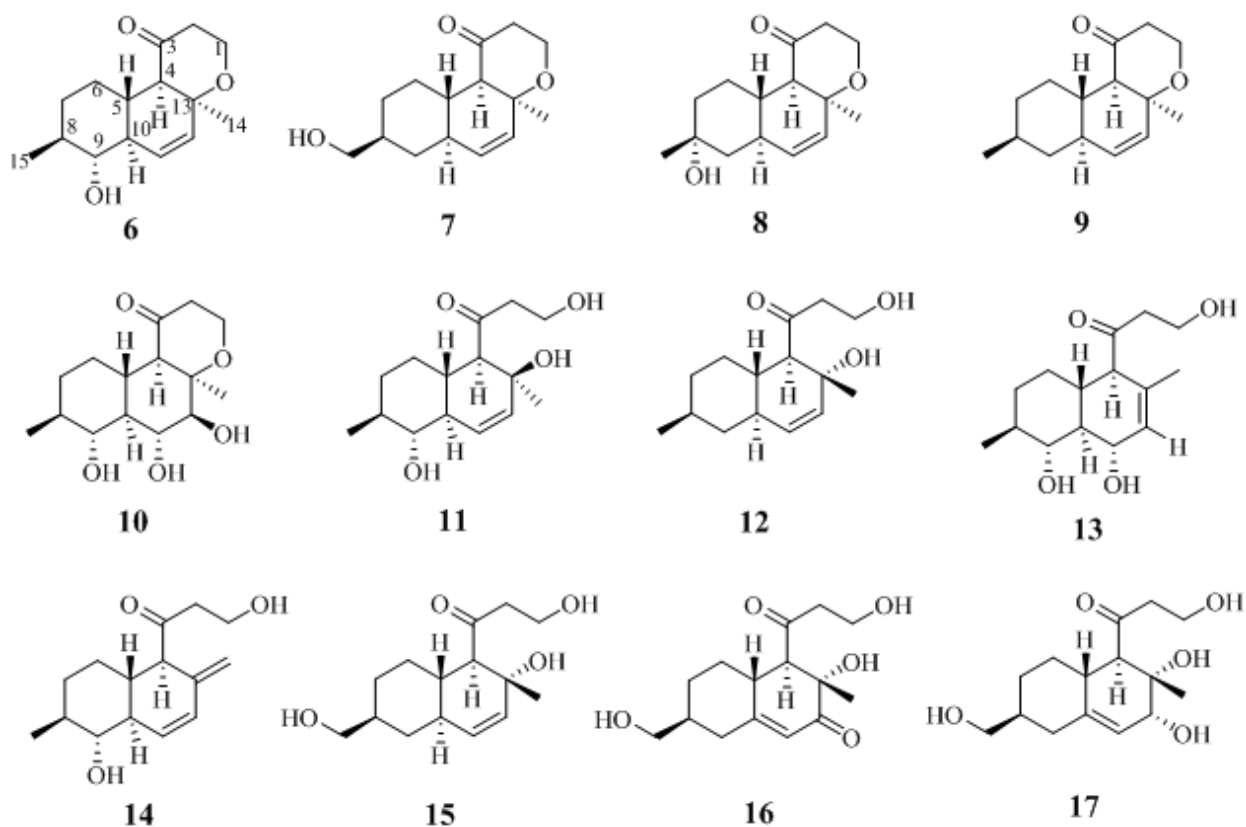


Figure 2. Chemical structures of zosteropenillines A-L (6-17)

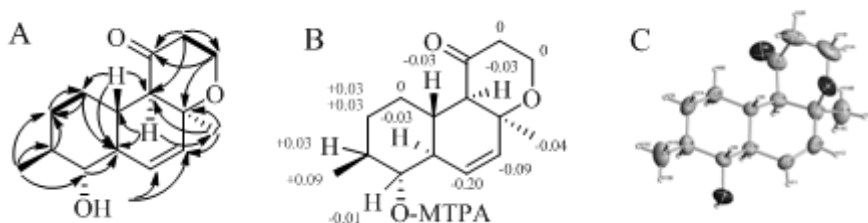


Figure 3. (A) Key HMBC and COSY correlations of **6**; (B) $\Delta\delta(\delta_S - \delta_R)$ values (in ppm) for the (S)- and (R)-MPTA esters of **6**; (C) Crystal structure of **6**

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Oral Presentation

DEPHOSPHORYLATION OF LIPOPOLYSACCHARIDES BY ALKALINE PHOSPHATASE FROM MARINE BACTERIUM

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The recombinant alkaline phosphatase from the marine bacterium *Cobetia amphilecti* KMM 269 (CmAP) with a higher activity ($\geq 12,000$ U / mg) among the known analogues was previously biochemically characterized [1]. However, the biological role of the extracellular highly active alkaline phosphatase (AP) of the marine bacterium remains unknown. In addition, the *C. amphilecti* KMM 296 genome has been found the presence of several genes encoding alkaline phosphatases, probably associated with still unexplored functions for the marine life style [1]. The recent discovery of new properties and biological functions of AP from various sources shows the prospect of using them as drugs for various purposes of medicine and biotechnology [1,2]. Thus, intestinal APs was able to dephosphorylate lipopolysaccharides (LPS, endotoxins) of bacteria that resulted in a decrease of the overall inflammatory process [2]. It is known that LPS are major components of the cell envelope of Gram-negative bacteria, which are an important contributing factor to septic shock, in general, and Gram-negative septic shock, in particular. The endotoxic properties of LPS depend on the structural features of lipid A, a phosphoglycolipid. Currently, Gram-negative sepsis and endotoxic shock are a serious clinical problem. They give a high percentage of deaths even in countries with a developed health system. Modern medicine does not have specific and effective drugs for anti-endotoxin therapy. One of the approaches used to reduce the toxicity of LPS is dephosphorylation of lipid A. The cleavage of a single phosphate group in lipid A by mild acid hydrolysis of LPS has been shown to cause a significant weakening of its pyrogenicity and toxicity [3]. In this work, we first studied the effect of CmAP on the lipopolysaccharides *Escherichia coli* S-LPS-055: B5 and Ra-LPS-EH100 (Sigma). The total content of phosphorus in the initial solutions of LPS and after their treatment with AP followed by dialysis against water to remove free phosphate groups was determined by the method described [4]. It was found, that CmAP exhibits enzymatic activity against LPS, which largely depends on the aggregate state of the LPS molecules. The greatest activity of the enzyme removing up to 90% phosphorus was observed with LPS in the monomeric form. For complete dissolution of LPS, parameters such as sample concentration, buffer composition and pH, as well as the incubation temperature, were selected.

Thus, new data on the dephosphorylating activity of *CmAP* against LPS are a promising basis for developing a new therapeutic approach with the use of alkaline phosphatase for neutralizing the effects of bacterial endotoxins (sepsis, endotoxic shock).

The work was supported by the program “Far East”, № 18-4-051.

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*Oral Presentation***COMPARATIVE GENOMICS OF *ZOBELLIA*: ANALYSIS OF POLYSACCHARIDE LYASES GENES AND OPERONS****N.U. Chernysheva¹, G.N. Likhatskaya¹, O.I. Nedashkovskaya¹, M.P. Isaeva^{1,2}**¹*G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*²*Far Eastern Federal University, Vladivostok, Russia*

Marine flavobacteria are well-known microorganisms applied in bioconversion of complex organic materials into bioactive substances. These bacteria are a valuable source of hydrolytic enzymes, such as polysaccharide lyases that are promising for effective algal biomass utilization [1]. *Zobellia galactanivorans* is one of the most prominent and well-studied species in terms of algal carbohydrates degradation [2]. Therefore, genome investigation of the other representatives of the genus *Zobellia* has good prospects for the discovery of new commercially relevant enzymes.

We sequenced genomes of two flavobacteria *Zobellia amurskyensis* KMM 3526^T and *Zobellia laminariae* KMM 3676^T, which were isolated from seawater and brown algae samples [3]. The genomic evaluation of the genus *Zobellia* for hydrolytic potential reveals that the genomes contain numerous genes of carbohydrate active enzymes. Half of them occur separately in the genomes, and others are localized within clusters comprising additional transporter genes. Both flavobacteria contain up to 19 putative alginate lyases. Genomes encode a complex alginolytic system that provides a stepwise depolymerization of alginates and assimilation of products into the central metabolism. Detailed phylogenetic and comparative analysis of alginate lyases was performed. It was found that PL7 alginate lyases are duplicated and refer to two subfamilies, one of them is novel. The results of computer modelling suggested that the alginate lyases have different modes of action.

Sequencing and analysis of the genomes revealed a huge potential for polysaccharide bioconversion, including alginate catabolism. Specific alginate lyases of PL7 family can be used for potential application in biotechnology and industry.

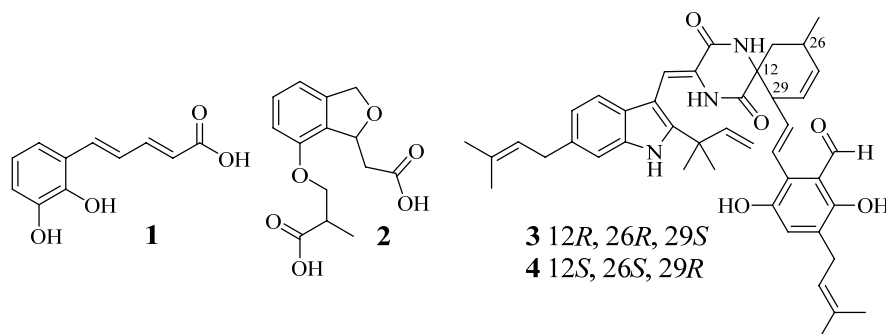
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*Oral Presentation***AUROGLAUCIN AND ECHINULIN RELATED METABOLITES FROM VIETNAMESE MARINE SEDIMENT-DERIVED FUNGUS *EUROTIIUM NIVEOGLAUCUM*****E.V. Ivanets, A.N. Yurchenko***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

The marine fungi are promising sources of bioactive compounds, which are usually have a cytotoxic, antibiotic and anti-inflammatory activities [1]. Since marine environment possesses complicated conditions for living, marine fungi can produce compounds with unique structure. Recently cephalosporin C was the only marine fungal compound that used as a medical drug, but today fingolimod developed from myriocin is approved by FDA and EMA, and plinabulin, the synthetic derivative of diketopiperazine phenylahistin, is under phase III of clinical trials (according to clinicaltrials.gov) [2, 3]. The coast of Vietnam is an insufficiently studied area of South China Sea, because most researches are focus on the coast of China and Taiwan Strait. Besides the high level of bacterial colonization in tropical seas is a stimulating factor for marine fungi. Therefore, investigation of fungi of Vietnamese coast is topical.

In this research work the metabolite composition of the marine-derived fungus *Eurotium niveoglaucum* (sediments, Nha Trang Bay, South China Sea) was investigated. As a result, two new polyketides niveoglaucines A and B (**1-2**) together with a number of known metabolites such as (+)-cryptoechinulin B (**3**) and (-)-cryptoechinulin B (**4**), flavoglaucin, tetrahydroauroglaucin, isodihydroauroglaucin, 5-hydroxy-6-(3-methylbut-2-enyl)-2-(pent-1-enyl)benzofuran-4-carbaldehyde, echinulin and neoechinulin were isolated. The structures of isolated compounds were established by combination of 1D and 2D ¹H and ¹³C NMR and HR ESIMS data, as well as ECD spectra.



It should be noted, it is the first case of isolation of (+)-cryptoechinulin B (**3**) and (-)-cryptoechinulin B (**4**) as the individual compounds [4]. Besides niveoglaucine B (**2**) have a

unique fragment of 3-hydroxyisobutyric acid (3-HIBA). To the best of our knowledge, there are no published natural compounds contained this moiety.

The possible biosynthesis scheme of niveoglaucines A-B (**1-2**) was proposed.

The study was supported by RFBR grant № 18-34-00621 mol_a.

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*Oral Presentation***OLIGO-ARGININE PEPTIDES AS A NEW CLASS OF LIGANDS OF NICOTINIC ACETYLCHOLINE RECEPTORS****I.E. Kasheverov, E.V. Kryukova, I.A. Ivanov, D.S. Lebedev, E.N. Spirova, D.A. Senko, N.V. Egorova, V.I. Tsetlin***Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*

Many peptide ligands of nicotine acetylcholine receptors (nAChRs) contain in their sequences a large number of positively charged amino acid residues. Among the most famous are waglerins and azemiopsin from the snake venoms as well as various alpha-conotoxins from the venom of the marine *Conus* mollusks, such as conotoxins RgIA and GeXIVA which are considered as potential analgesics. The latter contain 4 and 9 arginine residues, respectively. Moreover, our experience with conotoxins has shown that in many cases the introduction of a positive charge into the structure of a naturally-occurring peptide can significantly improve its affinity for distinct nAChR subtype. In particular, the analogue of [D12K]SIA showed 2 orders of magnitude higher efficacy for the muscle-type nAChR compared to the native α -conotoxin SIA [1], and the introduction of Arg9 in the sequence of α -conotoxin PnIA significantly increased affinity for neuronal $\alpha 7$ receptor [2].

For better understanding of the properties of Arg-rich cholinergic peptides, we synthesized a series of oligo-arginines and studied their ability to interact with muscle-type *Torpedo californica* and neuronal $\alpha 7$ nAChRs in radioligand assay, electrophysiology and Ca^{2+} -imaging experiments. A direct correlation between the length of oligo-Arg peptide and its cholinergic activity has been identified, the highest measured potency being 69 nM for the 16-membered peptide towards the *T. californica* receptor. Thus, the analysis of Arg-rich cholinergic peptides has promoted the discovery of the oligo-arginines reported here as a novel group of nAChR antagonists, which can be a basis for target work on the designing modified oligo-arginine peptides with increased affinity and selectivity towards distinct nAChRs.

The work was supported by RFBR grant № 18-04-01366.

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*Oral Presentation***PROTECTIVE PROPERTIES OF HISTOCHROME IN EXPERIMENTAL STROKE BRAIN MODELS****Irina G. Agafonova***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

The problems of pathology of cerebral vessels in patients are the most socially significant. Cerebral changes are characterized by a high percentage of mortality among patients. Disability, rehabilitation after stroke, for example, leads to greater economic costs. Moreover, diseases of the blood vessels of the brain become more common among the young working-age people. Therefore, the search for new drugs and approaches to the treatment of these diseases is of great importance.

For many years, biological and pharmacological properties of HistoChrome as cardiac drug have been studying at G.B. Elyakov PIBOC FEB RAS. In this study we have expanded the range of HistoChrome effect on cerebrovascular diseases. Two independent experimental models of stroke (hemorrhagic and ischemic) were taken to study the effect of HistoChrome on brain changes. The model of hemorrhagic stroke was induced by brain cell injury in the rats. The ischemia of focal blood rat brain circulation was induced by permanent occlusion of middle cerebral artery.

Using the hemorrhage model, we have found that the disturbances of microcirculation, ischemic changes in neurons, and neutrophil infiltration in the surrounding hematoma tissue decreased under the therapy with HistoChrome. A positive effect was observed after injection of HistoChrome within 1 hour after the onset of the induction of pathology. The infiltration of neutrophils began 12-24 hours after the ischemia. We postulate that these changes together with edema and oxidative stress are potential targets for the treatment using the compound studied. In a case of applied ischemic model we have shown that HistoChrome reduces the risk of thrombosis in small-small arterioles. The area of ischemia in the bleeding tissue after occlusion is not growing in comparison with the control. This effect was verified by the method of Magnetic Resonance angiography.

The advantage of MR Imaging and Angiography are in performing the complete cycle of monitoring of the functional and morphological changes of the body animals from the norm to pathology and to the final reaction of the pharmacological effects of the studied compounds. The study was carrying out on the equipment of the Collective Usage Center of PIBOC FEBRAS, "Far East center of noninvasive radio-frequency diagnostics of biological objects" (FECNRFD).

The work was supported by FEB RAS grant No. 18-4-003.

*Oral Presentation***FIRST TWO-CHAIN PEPTIDE TOXIN FROM SEA ANEMONE *HETERACTIS CRISPA***

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Sea anemone venoms contain diverse toxins most of which target voltage-gated sodium channels (Na_v). More than fifty toxins of four distinct structural types have been isolated and characterized from different sea anemone species. All of the sea anemone toxins are expected to bind with site 3 of the Na_v inhibiting channel inactivation alike to scorpion α-toxins and spider δ-toxins do regardless of their different folds. Despite many toxins have been isolated, a limited number of toxins were investigated in terms of ability to discriminate between closely related Na_v subtypes.

In this work, two known type II toxins, RTX-III and δ-SHTX-Hcr1f (= RpII), as well as new, RTX-VI, were isolated from 20% aqueous-ethanol fraction of *Heteractis crispa*. Toxin RTX-VI turned out to be an unusual natural analog of RTX-III consisting of two, not one, disulfide-linked peptide chains and was devoid of Arg13 which is important for the selectivity and affinity of such peptides to Na_v channels. At the same time, CD spectra of three peptides substantially overlapped indicating an identical conformation of these toxins. Electrophysiological screening of *Heteractis* toxins on nine Na_v channel isoforms revealed their different selectivity. Obtained data demonstrate that δ-SHTX-Hcr1f and RTX-VI toxins modulate insect (BgNa_v1, VdNa_v1) and CNS (Na_v1.1-1.3, Na_v1.6) Na_v but not skeletal muscle (Na_v1.4) or PNS (Na_v1.8) channels. Among three investigated toxins, only RTX-III affected cardiac Na_v1.5. The absence of Arg13 in RTX-VI structure abolish its effect on the Na_v1.5 and Na_v1.3 currents, however, this does not prevent toxin binding with Na_v1.6 regardless of efficacy decrease. Moreover, distinct from RTX-III, RTX-VI was shown to modulate Na_v1.2 of CNS. Thus, *H. crispa* type II toxins could be used as molecular tools for studying of some subtypes of mammalian or insect Nav channels rather than as pharmacological agents.

The work was funded by RFBR according to the research project № 18-04-00631.

Oral Presentation

THE SECONDARY METABOLITES FROM THE MARINE-DERIVED FUNGUS *ASPERGILLUS FLOCCULOSUS*

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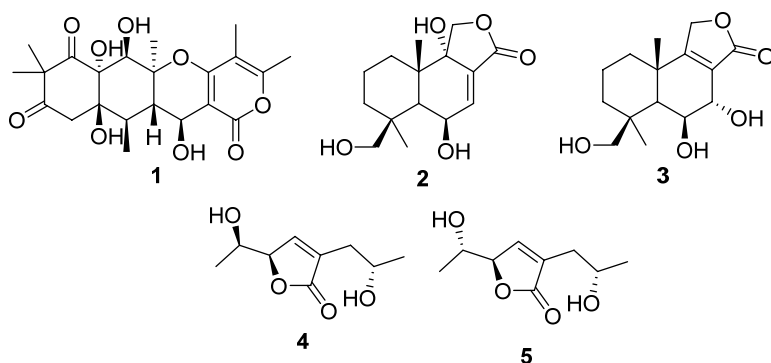
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Marine-derived fungi is one of the most promising source of biologically active compounds. Tropical waters with high bacterial diversity are good conditions for developing of bioactive metabolites producers [1].

The fungus *Aspergillus flocculosus* was isolated from the sediment sample, which collected in Nha Trang bay (Vietnam). The careful chromatographic separation with silica gel, sephadex LH-20 and following normal and reversed phase HPLC purification yielded 10 individual compounds. The structures of isolated compounds were established by 1D and 2D NMR spectroscopy and high-resolution mass spectrometry as new meroterpenoid 12-epi-aspertetranone D (**1**) and its known epimer aspertetranone D [2], two new sesquiterpenoids **2** and **3** and their known *p*-nitrobenzoyl derivatives [3], new tetraketide aspilactonol G (**4**) and two its known isomers aspilactonol A (**5**) and dihydroaspyrone, as well as known diketopiperazine mactanamide[4]. The absolute configurations of all stereocentres of **4** and **5** were confirmed by modified Mosher's method.



The cytotoxicity of isolated compounds against human prostate cancer cell lines and murine neuroblastoma cell line was evaluated. In addition, the neuroprotective effect of these compounds in 6-hydroxydopamine model of Parkinson's disease was studied.

The study was supported by RFBR grants № 18-34-00621 (chemical study) and № 18-34-00737 (bioassays).

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*Oral Presentation***THE NEW *HETERACTIS MAGNIFICA* KUNITZ-PEPTIDE INTERACTS WITH SERINE PROTEASES****A.N. Kvetkina¹, L.A. Kaluzhskiy², E.V. Leychenko¹, E.A. Zelepuga¹, M.P. Isaeva¹, A.S. Ivanov², E.P. Kozlovskaya¹**¹*G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*²*V.N. Orekhovich Institute of Biomedical Chemistry of the Russian Academy of Medical Sciences*

Proteolytic enzymes participate in all living processes, including food digestion, blood coagulation, hormone processing, apoptosis, and others. Deficiency in regulation of the significant molecules can result from the development of neurodegenerative, cardiovascular, and other disorders [1]. Therefore the investigation of protease inhibitors as protease activity regulators is the relevant task. Among the known protease inhibitors Kunitz-peptides are the most characterized due to their distribution in numerous living organisms [2]. They consist of about 60 amino acid residues and form the compact $\alpha+\beta$ structure, stabilized by three disulfide bonds [3]. The primary and first discovered function of Kunitz-peptides is an inhibition of serine proteases, but they also possess other biological activities, such as modulating of ion channel, exhibition of anti-inflammatory, antihistamine, antifibrinolytic, hemostatic activities, etc [2]. We investigated the interaction of Kunitz-peptide HMIQ3c1 of sea anemone *H. magnifica* with serine proteases by surface plasmon resonance method. This method permits to determine kinetic and thermodynamic parameters of protease-inhibitor complex formation. The results revealed a high affinity of HMIQ3c1 to trypsin (K_D 1.07×10^{-9} M), chymotrypsin (K_D 4.70×10^{-8} M), kallikrein (K_D 2.81×10^{-8} M), and elastase (K_D 1.11×10^{-7} M). Moreover, the peptide has been shown recently inhibited trypsin with K_i 5.0×10^{-8} M. The interaction of HMIQ3c1 with neutrophil elastase and kallikrein may indicate its anti-inflammatory activity. Thus, Kunitz-peptide HMIQ3c1 of *H. magnifica* makes strong enough complexes with serine proteases that allow us to consider it as potential pharmacological tool.

This work was partially supported by the Presidium of Far Eastern Branch of the Russian Academy of Sciences (grant number RUS_ST2017-228).

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*Oral Presentation***DEVELOPMENT OF CHEMICAL PROBES EXPLOITING QUINONE METHIDE CHEMISTRY FOR BIOCHEMICAL APPLICATIONS****Lee-Chiang Lo***Department of Chemistry, National Taiwan University, Taipei 106, Taiwan*

In the post-genomic era, development of small molecules as tools to help studying biological phenomenon has gained increasing attention. It is especially true in the development of activity-based probes (ABPs) due to their remarkable ability to label and enrich the designated enzymatic activities on protein extracts or living cells. For nearly two decades, my group has been devoted to the development of functional molecules and exploited these molecules in a wide variety of biochemical applications. Here I would like to present our results on the design, synthesis, and applications of ABPs for tyrosine phosphatases, glycosidases, and sulfatases [1-3]. A typical probe consists of four structural components; a recognition head, a latent trapping device, a linker, and a reporter group. The probes themselves are also the substrates of the corresponding hydrolases. Once the recognition head is cleaved by the target hydrolase, the probe will be selectively activated, leading to covalent modifications of the enzyme. Activity-based probes have versatile applications. For example, a sialidase probe could be utilized to capture influenza viruses [4], while a fucosidase probe could be used to visualize and locate lysosomal α -L-fucosidase activity in cells [5]. In addition, we took advantage of the featured chemoselective reaction between arylboronic acids and hydrogen peroxide to develop probes for H₂O₂ [6,7]. This was the pioneering work in this field.

The work was supported by the Ministry of Science and Technology grant MOST106-2113-M-002-005.

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*Oral Presentation***THE DEVELOPMENT AND CHARACTERISATION OF NOVEL LIGANDS TO ASICS****Sergey Kozlov***Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences*

Acid sensing ion channels (ASICs) are a most important cellular sensor to extracellular media acidification. The tiny reduction of pH level can be converted into influx of sodium and calcium ions through these channels following the depolarization of cell membranes. In mammals 4 genes coded structures of these ion channels but the principal importance has ASIC1a subtype widely distributed in brain and ASIC3 subtype expressed predominantly in peripheral neurons. ASICs are implicated in pain, neurological and psychiatric diseases but their therapeutic potential is limited by lack of selective ligands. We search ligands to ASICs in different herbal extracts, since medicinal plants can produce compounds to many cellular targets. As a result, one alkaloids' inhibitor of ASICs was purified from thyme *Thymus armeniacus* while another alkaloid with activating effect on ASIC3 was purified from *Laurus nobilis* leaves. The analysis of structural features of both alkaloids results in a determination of the structural core necessary for an interaction with ASICs that presents also in endogenous isoquinoline alkaloids tetrahydropapaveroline and reticuline. It is interesting but these molecules are involved in pain relief in mammals via endogenous morphine biosynthesis pathway. At physiological pH endogenous isoquinoline alkaloids is able to effectively activate hASIC3 and rASIC3 and prevent steady state desensitization of these channels. *In vivo* experiments shown the analgesic effect in CFA induced hyperalgesia animal test for both inhibitor and activators of ASICs, so the role of ASICs in the nociception process is ambiguous and requires a more detailed study.

This work was supported by Russian Science Foundation grant No 18-14-00138.

*Oral Presentation***STRUCTURAL BIOINFORMATICS IN THE STUDY OF COLD-ACTIVE ENZYMES FROM MARINE ORGANISMS**

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Cold-active marine enzymes constitute an attractive resource for biotechnological applications. Some marine enzymes are important analytical tools. Enzymes from marine organisms with unique properties have been studied at the Pacific Institute of Bioorganic Chemistry: DNase from hepatopancreas of Kamchatka crab, specific for double-stranded DNA, alkaline phosphatase from marine bacteria, with the highest specific activity among known phosphatases, endo-glucanases of marine mollusks, showing transglycosylation activity and capable of synthesizing biologically active oligosaccharides, O-glycoside hydrolases from marine bacteria, modifying antigens of the blood group A and B. The spatial crystal structure of these enzymes is not currently established. Methods of structural bioinformatics were used for predicting three-dimensional (3D-) structure models of protein molecules from amino acid sequences. Fold recognitions of marine enzymes were carried out using 3D-PSSM, FUGUE and PHYRE servers. It was found that marine enzymes have folds with 100% confidence to enzymes having known crystal structures. Homology models of enzymes were generated by SWISS-MODEL, ModBase, I-TASSER servers and homology model module of program MOETM. The full-length structure models were constructed for nucleases duplex-specific nuclease (EC 3.1.30.2) from *Paralithodes camtschaticus* (Q8I9M9), S1/P1-type nuclease (EC 3.1.30.1) of marine fungus *Penicillium melinii* (D3JY17), endo-1,3-beta-D-glucanases (EC 3.2.1.39) of marine bacteria *Formosa algae* (A0A0B5GQL6) and from marine mollusks *Littorina sitkana* (C0KUK2), *Perna viridis* (B9W0H7), *Pseudocardium sachalinensis* (Q7Z0T2), *Chlamys albidus* (Q4FCS2), *Chlamys rosea* (Q4FCS1), *Mizuhopecten yessoensis* (Q5I6N3), alkaline phosphatase (EC 3.1.3.1) from marine bacterium *Cobetia marina* strain KMM 296 (Q1W622), alpha-galactosidase (EC 3.2.1.22) of the marine bacterium *Pseudoalteromonas* sp. KMM701 (Q19AX0), alpha-N-acetylgalactosaminidase (EC 3.2.1.49) of the marine bacterium *Arenibacter latericius* KMM 426T (E1AXI3) and alginate lyases (EC 4.2.2.-) family PL7 of the marine bacterium *Zobellia* sp.

Marine enzyme models were used for active sites analysis, *in silico* mutagenesis and for prediction of the enzyme-substrate and enzyme-inhibitor complexes structures by molecular docking approach. Models of complexes were building with programs GRAMM1.03, Docking module of MOE and Autodock4. Atomistic details of marine enzymes active sites, substrate binding subsites, inhibitors and metal binding sites were obtained. A comparison of the structure of psychrophilic marine enzymes and thermophilic enzymes was carried out by simulation molecular dynamics at various temperatures using the equipment of Shared Resource Center "Far Eastern Computing Resource" IACP FEB RAS (<https://cc.dvo.ru>). Marine enzymes 3D-structural data will allow an understanding better of the structure–function relationships and regulation activities of the marine cold-active enzymes.

*Oral Presentation***THE FIRST PEPTIDE ASIC1A CHANNEL MODULATORS FROM SEA ANEMONE**

R.S. Kalina¹, I.N. Gladkikh¹, P.S. Dmitrenok¹, S.G. Koshelev², E.A. Zelepuga¹, S.A. Kozlov², E.P. Kozlovskaya¹, M.M. Monastyrnaya¹

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Pharmacology of natural peptide toxins and diversity of their biological effects is complicated and intensively studied problem. Currently, the most of investigations is aimed at both screening of biologically active natural compounds and searching for their interaction mode with pharmacologically perspective molecular targets to construct more effective synthetic analogs. It is known that acid sensing ion channels (ASICs) are expressed mainly in central (ASIC1a) and peripheral (ASIC3) nervous system; so their modulators are considered as neuroprotective or analgesic compounds, respectively. Interestingly, toxins isolated from spider and snake venoms modulate ASIC1a and ASIC1a-containing channels, in contrast to the sea anemone toxins selective for ASIC3 and ASIC3-containing channels.

In this work, three new peptides, π -AnmTX Hcr 1b-2, -3, and -4 (41 aa long), from tropical sea anemone *Heteractis crispa* were identified and characterized as the first ASIC1a modulators from sea anemone venom. A combination of Edman degradation and tandem mass spectrometry allowed us to attach them to the structural class 1b of sea anemone toxins. This class is also represented by Hcr 1b-1, APETx1 – APETx4, BDS-I – BDS-II, and crassicorin-I molecules with various selectivity for Na_v, K_v, and ASIC3 channels. Hcr 1b-2, -3, and -4 share 78–95% and 46–49% identity with ASIC3 modulators Hcr 1b-1 from *H. crispa* and APETx2 from *A. elegantissima*, respectively. A key element of APETx2 pharmacophore, Phe15-Tyr16-Arg17, for ASIC3 is vary in *Heteractis* toxins, Phe15-Met16-Leu17. All ASICs modulators are the basic peptides that substantially favors to their binding to negatively charged channels. Homology models of Hcr 1b-2, -3, and -4 toxins are different from their structural template, APETx2 (PDB ID: 1WXN), in term of surface charge distribution. Remarkably, distribution of positive electrostatic potential of Hcr 1b-4 is distinct from those of both APETx2 and other *Heteractis* toxins. These observations are in accordance with obtained IC₅₀ values of Hcr 1b-2, -3, and -4 for inhibition of ASIC1a.

The computational study of peptides was funded by RFBR according to the research project № 18-38-00387.

Oral Presentation **α -AMYLASE INHIBITORS ARE MAJOR COMPONENTS OF SEA ANEMONE *HETERACTIS MAGNIFICA* MUCUS****O.V. Sintsova, E.V. Leychenko, I.N. Gladkikh, M.M. Monastyrnaya, E.P. Kozlovskaya***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Sea anemones (phylum Cnidaria) are ancient sessile predators inhabiting the marine environment. Sea anemone mucus, due to its multiple and vital functions, is a valuable substance for investigation of new biologically active peptides. Pancreatic α -amylase inhibitors have been recently isolated from sea anemones [1]. They have a great pharmacological potential for the treatment of type II diabetes, which accounts for 90% of all diabetes cases. The aim of the work is a searching of new pancreatic α -amylase inhibitors in mucus of sea anemone *Heteractis magnifica*.

Compounds of *H. magnifica* mucus were separated by multistage liquid chromatography and resulting fractions were analyzed by MALDI-TOF MS. Peptide maps constructed according to the molecular masses and hydrophobicity showed presence of 326 both new and known peptides. Most fractions inhibited porcine pancreatic α -amylase, thus proteomic analysis revealed that α -amylase inhibitors along with proteinase inhibitors, pore forming toxins and neurotoxins are major components of *H. magnifica* mucus which play an important role in the successful existence of sea anemones. Magnificamide, the major α -amylase inhibitor of *H. magnifica*, was isolated and its amino acid sequence was determined. BLAST analysis of this sequence revealed only one sequence-based homolog (87.5%), which corresponded to helianthamide, α -amylase inhibitor from *Stichodactyla helianthus*. Magnificamide contains 44 amino acid residues; its molecular weight is 4770 Da.

The recombinant analog of magnificamide was obtained with the help of genetic engineering approaches. Artificial gene encoding the peptide was cloned into the pET32b vector, and expressed in *Escherichia coli* as part of a fusion protein. The fusion protein was isolated from the cell lysate by metal affinity chromatography, hydrolyzed by endoproteinase, and then the recombinant magnificamide was purified by RP HPLC. The average yield of the target peptide was 2 mg per liter of cell culture. It inhibited porcine pancreatic and human saliva α -amylase. Thus we obtained functionally active recombinant analog of magnificamide, which will be used for further study of its biological activity as potential drug for treatment of type II diabetes.

The work was partially supported by RFBR grant № 18-38-00389.

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PROPERTIES AND SUBSTRATE SPECIFICITIES OF ALGINATE LYASES FROM MARINE BACTERIUM *FORMOSA ALGAE* KMM 3553**A.A. Belik, A.S. Silchenko, O.S. Malyarenko, A.B. Rasin, M.I. Kusaykin***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Alginate lyases show antioxidant properties *in vitro*, inhibit growth and differentiation of adipocytes and absorption of saturated fatty acids, exhibit antiallergic properties by suppressing IgE and stimulate immune system by increasing of production of cytokines (G-CSF, TNF- α , interleukins) in murine macrophages, inhibit growth of osteosarcoma MG-63 cells by 60-70%. Alginate oligosaccharides have advances due to their low toxicity, availability of raw materials, environmentally safe production. Biological activity of alginates strongly depends on monosaccharide composition and polymerization degree that increases the importance of screening of suitable raw materials and main instruments of their modification.

We expressed recombinant forms of five alginate lyases of marine bacterium *Formosa algae* KMM 3553. By using the method of assaying of reducing sugars it was shown that these enzymes are active against polymannuronic, polyguluronic and mixed type of alginic acids. Five enzymes were classified as polymannuronate lyases (EC 4.2.2.3) and three as polyguluronate lyases (EC 4.2.2.11). Recombinant alginate lyase ALFA3 had been put under deeper research. Amino acid sequence of recombinant alginate lyase ALFA3 from polysaccharide-degrading marine bacterium *Formosa algae* KMM 3553 appeared to be 288 residues, molecular weight – 33.8 kDa. Enzyme was equally able to digest three types of sodium alginates (M-enriched, G-enriched and MG-mixed) with equally effectiveness according to reducing sugars assay: 21.5 U/mg for M- and MG- and 18.7 U/mg for G-. pH optimum was 6.0 for all types of substrates. Temperature optimum was estimated at 35 °C, half-inactivation happened at 42 °C for 30 min. Ions of Na⁺ in at least 0.1 M were essential for reaction proceeding, ions of K⁺ and Ca²⁺ in concentrations up to 0.5 M did not influence the activity. Km was calculated as 0.12 mM. While sodium polymannuronate was completely digested to oligosaccharides in 24 hours, G-enriched substrate remained significant high-molecular fraction and MG-substrate remained slight high-molecular fraction. These fractions had tendency not to disappear even after exhaustive digestion. ¹H NMR analysis of products showed that in the case of sodium polyM there appeared only H-4 (Δ M) signals (5.5 ppm) and H-1 (M) signal intensity decreased (4.7 ppm). In the case of polyG-enriched substrate the intensity of H-4 (Δ G) (5.8 ppm) signal was only slightly higher than of H-4 (Δ M) (5.5 ppm), there was visible accumulation of M-red and G-red reducing ends as well, but not exhausting of G1 and G5 signals. This gives the evidence that in all substrates ALFA3 is able to hydrolyze MM, MG and GM bonds, but not GG. Products of polymannuronate digestion in concentrations 200 μ g/ml inhibited anchorage-independent colony formation of human melanoma cells SK-MEL-5, SK-MEL-28, RPMI-7951 up to 17% stronger in compare with native polymannuronate. This fact supports previous data and proposes the potential of mannuronate oligosaccharides to take part in tumor synergic therapy.

The work was supported by RFBR Grant 18-04-00905.

*Oral Presentation***RED BLOOD CELLS: ION TRANSPORT, ROLE IN THROMBUS FORMATION, AND INTERACTION WITH ARTIFICIAL SURFACES AND NANOPARTICLES****Ingolf Bernhardt***Laboratory of Biophysics, Saarland University, P.O.Box 151150, 66123 Saarbruecken, Germany. Email: i.bernhardt@mx.uni-saarland.de*

The talk will focus on three topics of our investigations of red blood cells (RBCs).

(i) The identification and characterization of a so far unknown $K^+(Na^+)/H^+$ exchanger in the RBC membrane and its role in certain diseases, e.g. cryohydrocytosis.

We investigated the so called low ionic strength (LIS) effect on the residual K^+ and Na^+ fluxes of RBCs. This effect was under investigation for about 100 years. On the basis of experimental data as well as theoretical calculations we were able to demonstrate that the so far assumed residual monovalent cation fluxes are only possible to explain on the basis of a novel carrier mechanism.

(ii) The role of RBCs in thrombus formation.

In current models the contribution of RBCs in the process of thrombus (clot) formation is assumed to be purely passive. However, we were able to demonstrate that RBCs can play an active role in thrombus formation.

The enhancement of the intracellular Ca^{2+} content of RBCs induced by lysophosphatidic acid (LPA, substance released by activated thrombocytes) results in the exposure of phosphatidylserine (PS) on the outer leaflet of the cell membrane due to the activation of the scramblase. In addition, it leads to cells shrinkage due to the activation of the Ca^{2+} -activated K^+ channel and the resulting KCl loss.

To study whether the Ca^{2+} uptake of RBC results in an enlargement of the mechanical cell-cell interaction, measurements applying holographic optical tweezers as well as single-cell force spectroscopy have been carried out. It was possible to demonstrate that after LPA treatment in the presence of Ca^{2+} , a pronounced adhesion of the RBCs could be observed. In control experiments (without LPA) it was only possible to detect a weak interaction between the cells.

(iii) The interaction of RBCs with artificial, e.g. nano-structured, surfaces.

To study the interaction of cells with surfaces in real time a new method, the digital holographic microscopy, allowing a focus trekking of the cells during sedimentation will be presented. In addition, the effect of nanoparticles on the Ca^{2+} content and the intracellular pH of RBCs based on fluorescence microscopy and FACS measurements will be shown.

*Oral Presentation***ACTIVATION OF ANGIOGENESIS AND WOUND HEALING IN DIABETIC MICE USING •NO-RELEASING DINITROSYL IRON COMPLEXES****Yun-Ming Wang¹, Yu-Jen Chen,¹ Wen-Feng Liaw,² Shyng-Shiou F. Yuan³**

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In diabetes mellitus, abnormal angiogenesis due to endothelial dysfunction impairs the wound healing and stimulates the attempts to develop chemotherapies for the prevention of diabetic foot ulcers and mortality. In this study, activation of angiogenesis and wound healing by direct treatment of nitric oxide using •NO-delivery dinitrosyl iron complexes (DNICs) and the therapeutic effect in diabetic mouse were investigated. The *in vitro* and *in vivo* study demonstrates that **DNIC-1** [$\text{Fe}_2(\mu\text{-SCH}_2\text{CH}_2\text{OH})_2(\text{NO})_4$] features a sustainable •NO-release reactivity ($t_{1/2} = 27.4 \pm 0.5$ h) and displays the pro-angiogenesis activity overwhelming the other •NO donors and, in particular, vascular endothelial growth factor (VEGF). Moreover, **DNIC-1** rescues the impaired angiogenesis on hind limb under ischemia and accelerates the wound closure in diabetic mouse. This study highlights the sustainable •NO-release reactivity of the synthetic **DNIC-1** for the translation into a novel chemotherapy to replace VEGF and to activate of angiogenesis and wound healing in diabetes.

This work was supported by grants from the Ministry of Science and Technology, Taiwan (MOST 106-2113-M-009-023).

*Oral Presentation***SPR BIOSENSORS IN DIRECT MOLECULAR FISHING: IMPLICATIONS FOR PROTEIN INTERACTOMICS****A.S. Ivanov***Institute of Biomedical Chemistry, Pogodinskaya str. 10, bld.8, Moscow, 119121, Russia. E-mail: alexei.ivanov@ibmc.msk.ru*

There is increasing evidence that in living systems proteins exist and function within stable or dynamic molecular complexes [1]. Protein–protein interactions (PPIs) determining formation and lifespan of such complexes attract much interest; they are extensively studied by using various bioinformatic, genomic, and biochemical technologies [2–4]. In this context, biochemical methods are the most reliable ones: using these methods researchers investigate PPIs under conditions close to physiological.

Biochemical methods employ the strategy of molecular fishing for isolation of protein complexes and subsequent mass spectrometry identification of potential protein partners.

Molecular fishing is a variant of affinity-based isolation of target proteins from a lysate of the biological material due to specific interaction between the immobilized ligand (a bait molecule) and its putative (one or several) functionally competent partners (prey molecules) [4–6]. Various compounds have been used as the bait molecules; these include small organic molecules [7,8], proteins and nucleic acids [4].

In this report, we have summarized results of our studies on the use of SPR-based approach for direct molecular fishing of proteins from lysates of biological materials and identification of prey proteins by mass spectrometry [9-15]. We initially consider a general strategy for the use of SPR biosensors at the particular experimental stages of molecular fishing with special attention to the SPR-based optimization of experimental protocols (including immobilization of bait proteins on a carrier, evaluation of intactness of the immobilized bait protein, and optimization of protocols for preparation of tissue/cell culture lysates, etc.).

After that we consider the role of the SPR biosensor technology in the SPR-based analytical fishing. Finally, we demonstrate applicability of the SPR biosensor technology for analysis of ligand protein interactions using non-peptide small molecules as baits [16].

Acknowledgment: The author thanks the GE Healthcare (Russia) for scientific and technical support.

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*Oral Presentation***INFLUENCE OF RED ALGAL POLYSACCHARIDES ON NEUTROPHILS ACTIVATION, CYTOKINE SYNTHESIS AND INTESTINAL EPITHELIAL CELLS *IN VITRO*****E.V. Sokolova, A.O. Kravchenko, V.N. Davydova, A.S. Kuz'mich, N.P. Mishchenko, I.M. Yermak***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Red algal sulphated polysaccharides - carrageenans - are widely used in food industry due to their physical and chemical properties; however they also have numerous biological activities. The following carrageenan structural types isolated from red algae collected from the Pacific coast were used in the study: κ -, κ/β -, ι/κ -, and λ -types. The study aim was to investigate *in vitro* immunomodulatory properties of carrageenans with various structural types. These properties of algal polysaccharides alone and in combination with lipopolysaccharide (LPS) were investigated by means of changes in neutrophils size and granularity and the synthesis of cytokines in blood *in vitro*. The influence on neutrophils' size and granularity correlating with the activation degree of these cells were measured by flow cytometry. The data revealed that carrageenans alone did not affect the former but increased the latter. Further, a combined action of these polysaccharides with *E.coli* LPS illustrated an inhibitory effect of carrageenans on the neutrophils' size. The level of granularity induced by LPS alone reserved unaltered by the addition of the polysaccharides. Carrageenans activated neutrophils with much less potency than LPS. Carrageenans especially with low contents of sulphate groups were able to interfere with LPS *in vitro* resulting in reducing neutrophils activation.

Carrageenans are also known as potent immunomodulators with remarked ability to cytokine synthesis [1]. IL-4 promotes T helper cell type 2 (Th2) differentiation and stability and inhibits Th1-cell differentiation. All structural types of carrageenans showed decreased IL-4 production in blood *in vitro* that is correlated with the literature data reported by Tsuji and co-authors [2]. The addition of LPS to the reaction medium did not result in significant changes.

The interaction between carrageenans and the gastrointestinal tract starts with the frontline host defence coating the gastrointestinal tract—a layer of mucus composed mostly of mucins secreted by intestinal goblet cells. HT-29 is a colorectal cancer cell line used as an *in vitro* model for the intestinal epithelium because it retains many biochemical and physiologic features of the lower small intestine and ascending colon and can produce higher levels of mucins than other cell lines [3]. Our purpose in the study described here was to determine the protective action of carrageenans, alone and in combination with LPS, on survival of human epithelial monolayers of HT-29 cells treated with ethanol. All of the carrageenans investigated were inert in response to intestinal epithelial HT-29 cells under normal conditions, and the detrimental effect of ethanol on the state of these cells provided an opportunity to assay the protective properties of red algal polysaccharides. Low sulphate content and the presence of the 3,6-anhydrogalactose in κ/β -carrageenan were prerequisites for restoration of human intestinal epithelial HT-29 cells by carrageenans.

Due to its high ability to adsorb water, carrageenan can improve drug dissolution and thus increase the oral bioavailability poorly water soluble drugs. Echinochrome (Ech) is the water insoluble active substance in the cardioprotective drug HistoChrome®, produced in Russia.

Carrageenans matrixes had been used to incorporate of Ech in order to study the effect of the polysaccharide on the Ech properties [4]. The effect of Ech alone and in carrageenans complexes on HT-29 cells treated with Ethanol was investigated. Ech also possessed an ability to restore HT-29 cells after exposure to ethanol and its activity retained in complexes with κ/β -carrageenan.

The work was supported by the RFBR grant № 18-34-00454 and the experiment with Ech by the RScF grant № 16-14-00051.

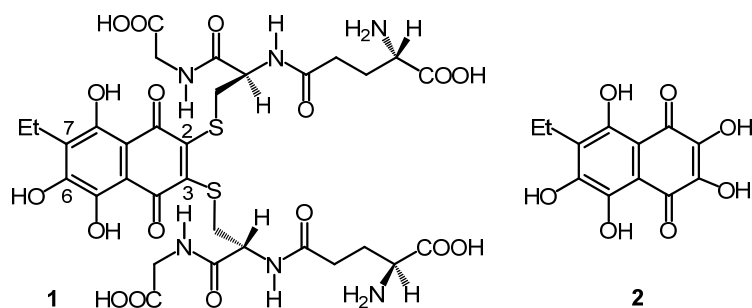
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*Oral Presentation***ELECTROCARDIOGRAPHY STUDY OF DIGLUTATHIONYL ANALOG OF ECHINOCHROME ON ADRENALINE-INDUCED MYOCARDIAL ISCHEMIA IN MICE****A.E. Zakirova, I.G. Agafonova, V.Ph. Anufriev***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Cardiovascular diseases are the most common diseases and occupy a leading position in the world mortality statistics. This fact is due primarily to the widespread coronary heart disease. For this reason, creation of drugs having cardioprotective action remains a topical task. Synthesis and studying of the compounds possessing potential cardioprotective properties are carried out in G.B. Elyakov Pacific Institute of Bioorganic Chemistry of Russian Academy of Sciences. Hydroxynaphthazarins belong to this group of compounds. Previously, the high cardioprotective activity of these compounds in *in vivo* experiments, comparable with echinochrome, was demonstrated. [1].

Present research aim was studying of influence 7-ethyl-2,3-diglutathionyl-6-hydroxynaphthazarin (DGE, **1**) on adrenaline-induced myocardial ischemia (MI) in CD-1 mice.



Experiments with animals were carried out on mature CD-1 mice of both sexes, 8-10 weeks of age, weighing 20-22 g. MI was induced by a single subcutaneous injection of 0.1% adrenaline hydrochloride solution at a dose of 5 mg/kg. Histochochrome (echinochrome **2** is active substance) at a dose of 1 mg/kg was used as a reference drug. Changes in the electrical conductivity of the heart were recorded by ECG. Before recording the ECG, the mouse was anesthetized using a solution of xylazine at a dose of 27 mg/kg animal weight. Electrocardiogram data were registered from limb lead II with the ECG recorder (Small Animal Instruments, USA). Electrocardiogram data was fixing for 1 hour. It was revealed that a steady electrocardiogram changes were recorded in 15 min after adrenaline injection. P peak and ST magnitude, RR and QT intervals, heart rate significantly changed relative to control data. Other ECG parameters such as P wave width, interval PR, R peak magnitude and QRS complex width

remained invariable. DGM subcutaneous injection in doses of 10, 5 and 1 mg/kg was carried out 15 min after adrenaline injection. The effectiveness of DGE was assessed by its ability to return parameters altered by adrenaline to intact parameters, especially ST and QT data, because they indicate ventricle repolarization changes. It was shown that in 15 minutes after adrenaline, DGM injection in a dose of 1 mg/kg significantly reduced QT interval duration, but did not reduce increased ST segment. As for DGM in doses of 10 and 5 mg/kg, the values of both parameters were reduced, which indicates the ventricular repolarization time normalization.

Thus, it is shown that 7-ethyl-2,3-diglutathionyl-6-hydroxynaphthazarin exerts a positive impact on dynamics of processes cardiac muscle recovering at mice with an induced myocardial infarction.

This work was partially supported by the FEBRAS grant No 18-4-003.

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*Oral Presentation***AUTOLOGOUS HINGE AS A UNIVERSAL ANTIBODY LOCK ENHANCE THE SELECTIVITY AND SAFETY OF ANTIBODY DRUG****Yun-Chi Lu¹, Chih-Hung Chuang², Wen-Wei Lin³, Tian-Lu Cheng^{1,4}**¹*Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*²*Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan*³*Department of Laboratory Medicine, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*⁴*Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung, Taiwan*

On-target toxicities caused by systemic administration of antibody drugs limit their application. Here, we “copied” the hinge region as a universal “antibody lock” and “pasted” it on the antigen binding site of the monoclonal antibody drug infliximab (Remicade) by linking with a disease-specific protease substrate to generate pro-Remicade which can be selectively activated at the disease site. The binding of pro-Remicade was 395-fold weaker than Remicade, and could be completely restored after activation with protease. The antibody lock also markedly prevented the neutralizing effect of an anti-idiotypic antibody induced by long-term drug administration to rheumatoid arthritis patients. In mice model, selectively activated pro-Remicade provided equivalent serum half-life and therapeutic efficacy comparable to Remicade while reducing on-target toxicities to maintain the immunity against *Listeria* infection. Moreover, the spatial-hindrance-based antibody lock was successfully applied to several antibodies. The universal antibody lock may revolutionize the development of antibody drugs to achieve disease site-specific therapy.

This work was supported by grants from the Ministry of Science and Technology, Taipei, Taiwan (MOST 106-2632-B-037-003).

Oral Presentation

CATIONIC HYBRID NANOPARTICLES FOR CANCER VISUALIZATION AND THERAPY

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Radiotherapy of oncological diseases is a powerful and effective method. But the inseparability of external, even focused, irradiation leads to severe side effects. In addition, high-energy gamma quanta are rapidly deactivated and cannot penetrate deeply into tissues. One way to improve the effectiveness of radiotherapy can be reduction of the energy of photons when interacting with large-diameter nuclei, such as tantalum [2]. Targeted delivery of drugs to the therapy area can be carried out using nanoparticles bearing a surface positive charge [1,3]. In addition, the use of an external magnetic field can be used to accumulate magnetic nanoparticles in a certain region [4].

The purpose of our study was to obtain hybrid nanoparticles (NPs) based on magnetic ferroxide as a core and a radiomodifying shell layer of tantalum oxide, which carry a surface positive charge.

NPs were synthesized in a multi-stage method. At the first stage, a Fe₃O₄ core was prepared by coprecipitation from ferrous sulfate (1 mol) and ferric chloride (2 mol) in the presence of citric acid (0-10 mol) as a stabilizer and excess aqueous ammonia at room temperature and vigorous stirring. After boiling for half an hour, the reaction mixture was subjected to hydrothermolysis at 180 ° C for a minimum of 12 hours. All the preparations obtained were washed from unincorporated compounds with distilled water and finally dried by lyophilization.

To prepare the hybrid core/shell NPs a solution of tantalum-hydrofluoric acid (0.2-1 mol), kindly presented by Professor M.A. Medkov (IC FEB RAS) was added to the suspension of ferroxide NPs in excess of aqueous ammonia and presence of citric acid (1-8 mol). After the hydrothermolysis NPs was purified and dried similarly as described above. The hybrid NPs obtained were characterized by dynamic light scattering, atomic force microscopy (AFM), and X-ray phase analysis. According to the results of elemental analysis, the final composition (core/shell ratio) of NPs is completely determined by the amounts introduced into the synthesis.

For the synthesis of cationic NPs, the surface modification of the nanoparticles was used 3-aminopropyl-triethoxysilane (APTES) in an alkaline medium. To obtain NPs with different charge density, a different amount of reagent was introduced into the reaction (Table 1). In addition, surface modification with polyethyleneimine and hexamethylenediamine was carried out by activation of the carboxyl groups of anionic NPs by using of a carbodiimide method and binding with the amine groups to form an amide bond. As can be seen from the Table, the the positive charge density of NPs can be varied by the concentration of APTES introduced into the synthesis. The use of polyamines leads to NPs with a much lower potential, but NPs with high molecular weight and high-branched polyethyleneimine (25 kDa) should interact with the cell surface preferably.

Table1. Size and Z-potential of cationic nanoparticles

NPs (ratio in moles)	Dimension, nm (%)	Zeta potential, mV
Fe/Ta/APTES (1/1/0,02)	107 (93,0)	13,9
Fe/Ta/APTES (1/1/0,04)	107(100,0)	17,8
Fe/Ta/APTES (1/1/0,08)	125 (91,9)	21,8
Fe/Ta/APTES (1/1/0,16)	120 (79,5)	27,5
Fe/Ta/ polyethyleneimine (1/1/0,09)	388 (100,0)	6,4
Fe/Ta/ hexamethylenediamine (1/1/0,09)	204 (100,0)	4,4

The X-ray analysis of NPs synthesized by coprecipitation at room temperature showed the absence of magnetite and the presence of amorphous oxides and hydroxides. The hydrothermal treatment method was used to recrystallize them. NPs obtained have excellent magnetic properties; its purification was greatly simplified. The cationic hybrid NPs possess good X-ray and NMR contrast properties as previously obtained anionic hybrid nanoparticles. To study acute and chronic toxicity NPs were introduced to CD-1 mice at different doses by intraperitoneal injection. The spontaneous deaths were not noted after 30 days. Morphological changes in brain, liver, kidneys, spleen and lungs were not observed according to the histological analysis. Thus, it is established that NPs obtained are not toxic at a dose of 200 mg/mouse and below.

To study the diffusion of nanoparticles from the zone of intramuscular injection in the absence of a magnetic field, the anionic NPs (1 mg/mouse) showed the disappearance of the drug

on the second day according to X-ray tomography. The magnetic NPs were detected for 24 days after using an external magnetic field.

Thus, a technology has been developed for the production of cationic hybrid nanoparticles based on iron and tantalum oxides, which carry a positive charge of different density on their surfaces. The use of such NPs is very promise for local radiotherapy and visualization of the resection zone of the neoplasm in humans.

The work was supported by Program of FEB RAS “Far East”, grant 18-3-042

Keywords: cationic nanoparticles, X-ray and NMR contrast, magnetic properties

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*Oral Presentation***PEPTIDE THERAPY FOR THE PREVENTION OF ALZHEIMER'S DISEASE****Rita Pei-Yeh Chen***Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan*

Alzheimer's disease (AD) is the most common neurodegenerative disease. Imbalance between the production and clearance of amyloid β ($A\beta$) peptides is considered the primary cause for the pathogenesis of AD. In this study, a peptide combining polyarginines (PolyR) (for charge repulsion) and a segment derived from the core region of $A\beta$ amyloid (for sequence recognition) was designed. The efficacy of the designed peptide, R8- $A\beta$ (25-35), on amyloid reduction and cognitive ability improvement was evaluated using the APP/PS1 double transgenic mice. Daily intranasal administration of PEI-conjugated R8- $A\beta$ (25-35) peptide significantly reduced $A\beta$ amyloid accumulation and ameliorated the memory deficits of the transgenic mice. Intranasal administration is a feasible way in peptide delivering. The modular design combining polyR and aggregate-forming segment produced a desirable therapeutic effect and could be easily adopted to design therapeutic peptides for other proteinaceous aggregate-associated diseases¹.

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THERAPEUTIC APPLICATION OF MSC-DERIVED EXOSOME**Woochul Chang***Department of Biology Education, Pusan National University*

Over the last decades, mesenchymal stem cells (MSCs) have been extensively studied with regard to their potential applications in regenerative medicine. MSCs possess the unique potential for use in cell-based therapy of heart diseases, especially in ischemic heart disease. The therapeutic potential of MSCs in myocardial regeneration is based on the ability of MSCs to directly differentiate into cardiac tissue and on the paracrine actions of factors released from MSCs. The predominant mechanism by which MSCs participate to tissue repair is through a paracrine activity. Via the production of a multitude of trophic factors with various properties, MSCs can reduce tissue injury, protect tissue from further degradation and/or enhance tissue repair. That is, the collected types of molecules released by the stem cells, called the secretome, or stem cell released molecules (SRM), number in the 100s, including proteins, microRNA, growth factors, antioxidants, proteasomes, and exosomes, and target a multitude of biological pathways through paracrine actions. Especially, exosomes have been identified as a new type of major paracrine factor released MSCs. They have been reported to be an important mediator of cell-to-cell communication. The diameter of exosomes ranges from 30 to 100 nm which contain an abundance of bioactive substances, such as mRNA, microRNA, and protein. In a myocardial infarction model, MSC-derived exosome had significantly better cardiomyocyte survival, enhanced capillary density, reduced cardiac fibrosis, and restored long-term cardiac function. These therapeutic effect of MSC-derived exosomes were mainly dependent on exosomal microRNAs. Taken together, MSC-derived exosome will be used for therapeutic delivery of miRNA targeted at cardiovascular disease.

The work was supported by NRF grant № 2017M3A9G7072568, 2018R1A2B6003158.

*Oral Presentation***THE NLRP3 INFLAMMASOME: AN IMPORTANT REGULATOR OF INFLAMMATORY DISEASES****Kuo-Feng Hua***Department of Biotechnology and Animal Science, National Ilan University, Ilan, Taiwan**E-mail: kuofenghua@gmail.com**URL: <http://ansc.niu.edu.tw/people/bio.php?PID=455>*

The NLRP3 inflammasome is a multiprotein complex composed of NLRP3, caspase-1 and ASC that plays a key role in the immune system. The NLRP3 inflammasome senses and can be activated in response to a highly diverse range of pathogens and environmental and endogenous danger molecules, such as *Escherichia coli*, nanoparticles of silicon dioxide, ATP, cholesterol crystals, uric acid crystals, amyloid- β and islet amyloid polypeptide. Although the NLRP3 inflammasome is important in innate immunity to fight infection, excessive activation of this complex is involved in a variety of common diseases, including atherosclerosis, type 2 diabetes, neurodegenerative diseases, gout and cancers. Therefore, the NLRP3 inflammasome activity and the associated signalling pathways are common targets for next-generation therapeutics. In this talk I will introduce the NLRP3 inflammasome mechanism of action, the role in diseases and therapeutics. I will also introduce the cell and animal models for studying the NLRP3 inflammasome and NLRP3-associated diseases. Additionally, I will show you some natural and synthetic compounds that can be used for ameliorating the NLRP3-associated complications. After this talk, I hope I have the opportunity to collaborate with you in investigating the NLRP3 inflammasome and its inhibitors in the near future.

The work was supported by Ministry of Science and Technology of Taiwan.

*Oral Presentation***THE ROLES OF A TRANSCRIPTION FACTOR ICE1 IN PLANT ABA-DEPENDENT SIGNALING PATHWAYS****Chien-Chih Yang, Ching-Shin Liang, Fa-Li Chang, Yi-An Lin***Department of Biochemical Science and Technology, National Taiwan University, Taipei, Taiwan*

To cope with ever-changing environment which is determined by temperature, light and water, plants employ a signal transduction network that enable the survival and growth. This signal network also monitor the status of growth and metabolism in the plants. The core of this network is usually comprised by a diverse of transcription factors that directly control the genes required for the plant responses. Some of the transcription factors can receive signals from more than one source. Both ABA-dependent pathways and ABA-independent pathways play roles in this stress response network [1]. We have studied the functions of a novel membrane protein, HHP1, from Arabidopsis. First, we identified HHP1 as a negative regulator in plant responses to osmotic stresses via an ABA-dependent pathway [2]. Further, the N-terminus of HHP1 was found to interact with a transcription factor, ICE1 [3]. Recently, we reported that ICE1 is involved in sugar responses in an ABA-dependent pathway [4]. Following to our discovery on the interaction of HHP1-ICE1, a similar interaction between HHP2/CAMTA3 or HHP3/ICE2 has been reported by another research group. This implied that such interaction between membrane protein and transcription factor should play a new molecular mechanism in plants.

The work was supported by MOST 104-2313-B-002-005, Taiwan.

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*Oral Presentation***ENZYMATIC BIOASSAYS USED FOR POLLUTION MONITORING OF MARINE ENVIRONMENT****A.V. Seytkalieva, N.I. Menzorova***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

The increasing environmental pollution and growing anthropogenic pressure on aquatic ecosystems make actual searching and developing easy-to-use and highly sensitive methods to test seawater quality. The highly sensitivity methods of physicochemical monitoring do not provide complete information on the content of complex pollutants in seawater. The other global approach for pollution monitoring is using because only the responses of living systems are able to integrate the various complex effects of contaminations. To assess environmental conditions in marine coastal waters the bioassays is widely applied using sea urchin gametes and embryos. The bioassay is sensitive to the presence of heavy metals, pesticides and detergents in seawater. "Sea Urchin Sperm Cell Toxicity Test" (SUSC test) has been developed and accepted as an official document for rapid evaluation of the toxic substances in wastewater and seawater [1].

We have developed a number of methods by using the sea urchin reproductive products to assess the effects of various contaminants in the aquatic environment. Our fluorescein diacetate (FDA) method is proposed to assay the nonspecific esterase activity of sea urchin sperm. The degree of fluorescence depends on the physical and metabolic states of the cell. It has been proved that the esterase activity can be reliable indicator of the toxic effects of pollutants.

Presently two our enzymes are unique due to to retain their activities in high salt solutions, included seawater, they isolated from the sea urchin *Strongylocentrotus intermedius* eggs. They are salt resistant alkaline DNase and phosphatase. Based on the enzymes we have developed highly sensitive express test systems to evaluate the level of seawater pollution in model experiments [2, 3].

All these bioassays , the SUSC-test, FDA method, DNase and phosphatase tests, were used for pollution monitoring of some water areas of the Japan (the Tumen River mouth; Troitsa bay, Peter the Great Bay; Ussuri and Amur Bays) and Okhotsk (east shelf of Sakhalin, near oil rigs and methane anomalies; the Sakhalin Gulf; and near the Kunashir island after the explosion at the nuclear power plant Fukushima) Seas both in natural surroundings and under anthropogenic impact.

Comparing the results of four test-systems has shown that the sensitivity of phosphatase test to general pollutions relatively similar to that of SUSC-test. The FDA method was the least

sensitive to the presence of pollutants. The phosphatase test appeared to be more sensitive to the presence of heavy metals and oil products in seawater than the DNase test. However the DNase was inhibited by a wider range of chemical compounds than phosphatase.

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MECHANISMS OF AUTOIMMUNE DISEASES DEVELOPMENT**Georgy A. Nevinsky***Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia*

Experimental autoimmune encephalomyelitis (EAE)-prone C57BL/6 mice are known as a model of human multiple sclerosis, while MRL-lpr/lpr mice is model of human systemic lupus erythematosus (SLE). Immunization MRL mice with DNA C57BL/6 mice with MOG, DNA-histones, and DNA-methylated bovine serum albumin (met-BSA) complexes results in similar effects on the bone marrow lymphocyte proliferation and differentiation profiles of hematopoietic stem cells as well as on the level of cell apoptosis in mice bone marrow and other organs. Anti-DNA antibodies are usually directed against histone-DNA complexes resulting from cell apoptosis. Immunization of EAE mice with MOG₃₅₋₅₅ results at acute phase of EAE development (7-20 days) in the production of catalytic antibodies hydrolyzing efficiently myelin basic protein (MBP), MOG, and DNA with parallel suppression of antibodies hydrolyzing histones. In contrast to MOG, immunization with histone-DNA results in the proteinuria suppression, a significant increase of the titers of antibodies against DNA, MBP, MOG as well as their catalytic activities in the hydrolysis of these antigens, but slightly changes the concentration of cytokines. In contrast to MOG, DNA-histone and DNA-met-BSA stimulated the formation of anti-DNA antibodies hydrolyzing DNA with a long delay (15-20 days). The data indicate that for C57BL/6 mice, different complexes of DNA with met-BSA and histones demonstrate antagonistic effects compared with MOG. DNA-histones stimulate the appearance of histones-hydrolyzing abzymes in the acute EAE phase, while with DNase activity only in significantly late period. The data shows that MOG, histone-DNA, and DNA-met-BSA have different effects on many bone marrow, cellular, immunological, and biochemical parameters of immunized mice, but all antigens finally significantly stimulate the development of the EAE. On the whole, the mechanisms SLE and EAE development have much in common and are clearly associated with the bone marrow differentiation profiles of hematopoietic stem cells and increase in lymphocyte proliferation, which leads to the production of catalytically active antibodies harmful for mammals.

Oral Presentation

NEUROPROTECTIVE ACTIVITY OF MARINE FUNGI METABOLITES IN TOXIN-INDUCED MODEL OF PARKINSON'S DISEASE

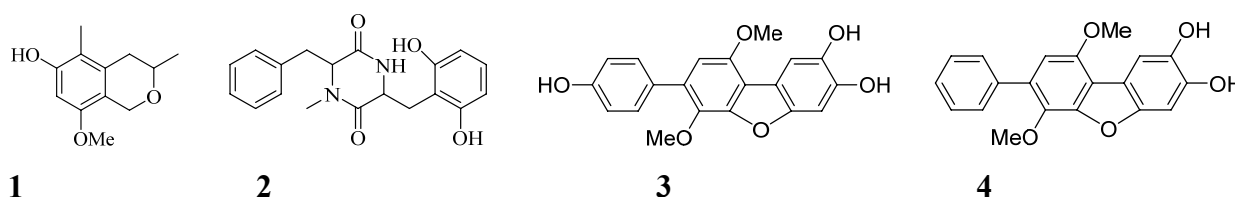
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Currently, Parkinson's disease is the most common neurodegenerative disease after Alzheimer's disease and no less socially significant disease, regardless of countries and regions, and requires an intensive search for new drugs that can prevent or contain the development of Parkinson's disease. Some metabolites of marine fungi show a neuroprotective effect in the *in vitro* models of Parkinson's disease. Thus, neoequinulin A, a known metabolite of a number of fungi of the genera *Aspergillus* and *Eurotium*, reduced the neurotoxicity of rotenone and 1-methyl-4-phenylpyridinium for PC12 cells [1].

The aim of our work was investigation of a number of metabolites isolated from the marine fungi *Aspergillus candidus* KMM 4676, *Eurotium niveoglaucum*, *Aspergillus flocculosus* and *Penicillium* sp. KMM 4672 in 6-hydroxidopamine-induced model of Parkinson's disease. Totally, the neuroprotective activity of 41 marine fungi metabolites was studied. Neuroblastoma Neuro2a line cells (1×10^3 cells/well) were treated with 50 μM of 6-hydroxydopamine (6-OHDA) during 1 h, after that neuroblastoma cells were cultivated with each investigated compound (10 μM) during 24 h. In other experiments, the substances were added to the cells 1 h before the addition of the neurotoxin. Viability of cells was measured by MTT assay.

Isochromene **1** [2] statistically significant increased the neuroblastoma cell viability on 11% (1h before 6-OHDA adding) and 14% (1h after 6-OHDA adding) compared with 6-OHDA-treated cells. Mactanamide **2** [3] increased the neuroblastoma cell viability on 18% compared with 6-OHDA-treated cells regardless of the time of substance addition. When the concentration of **2** was reduced tenfold this neuroprotective effect was preserved. Candidusine A **3** had no effect on viability of 6-OHDA-treated cells but its 4''-dehydroxylated analogue **4** [4] increased cell viability on more than 30% (1h before 6-OHDA adding) and 29% (1h after 6-OHDA adding).



Thus, same marine fungal metabolites protect neuronal cells from damaging effect of 6-hydroxydopamine.

The work was supported by RFBR grant № 18-34-00737.

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*Oral Presentation***SULFATED LIPOPOLYSACCHARIDES FROM MARINE GRAM-NEGATIVE BACTERIA: STRUCTURE AND BIOLOGICAL ACTIVITY****M.S. Kokoulin, A.S. Kuzmich, E.V. Sokolova, A.I. Kalinovsky, L.A. Romanenko***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Gram-negative bacteria are an important component of marine ecosystems where they occupy diverse habitats including deep-sea and hydrothermal vents, sea ice as well as open and coastal water areas. Lipopolysaccharides (LPS) are the major component of the outer membrane of Gram-negative bacteria. These characteristic and vital molecules maintain the contact between the bacterial cell and the surrounding environment; therefore, it is plausible that many of the functional changes induced by the harsh habitats can target LPS structure. One of the major interests to study marine Gram-negative bacteria is their ability to produce biologically active compounds, such as antibiotics, toxins, endotoxins (LPS), anti-tumor and antibacterial agents having pharmacological and biotechnological potential.

In the last few years, we have studied the O-polysaccharides (OPS) and lipids A of LPS from some marine Gram-negative bacteria that belong to genera *Cobetia*, *Idiomarina* and *Poseidonocella*. The chemical structure of the carbohydrate moiety of LPS of these marine Gram-negative bacteria is diverse and includes rare monosaccharides and non-carbohydrate substituents. Several new sulfated polysaccharides were found.

In detail, bacteria of genus *Cobetia* (*C. pacifica* KMM 3789^T and KMM 3878) produce sulfated OPS composed of trisaccharide repeating units. The type strain contains D-glucose 3-sulfate and D-galactose 3-sulfate. A distinctive feature of the OPS of KMM 3878 is the presence of d-galactose 2,3-disulfate. OPS of marine bacterium *C. litoralis* KMM 3880^T consists of trisaccharide repeating units and includes 2-keto-3-deoxy-D-manno-octanoic acid 5-sulfate. The same sugar residue was found in the disaccharide repeating unit of OPS from *Poseidonocella pacifica* KMM 9010^T. Besides eight-carbon sugar, this polysaccharide includes D-rhamnose 2-sulfate. The OPS of another bacterium of genus *Poseidonocella* – *P. sedimentorum* KMM 9023^T, also consists of disaccharide repeating units and contains 2-keto-3-deoxy-D-glycero-D-galactononic and D-glucuronic acid 2-sulfate. One more sulfated OPS was found in the LPS of deep-sea marine bacterium *I. abyssalis* KMM 227^T. It consists of pentasaccharide repeating units and includes 3-(4-hydroxybutyramido)-3,6-dideoxy-d-glucose 2-sulfate.

The LPS of bacteria of genus *Cobetia* were shown to exhibit a heterogenous lipids A, with hexa-, penta- and tetra-acylated species. Two penta-acylated lipid A species have been

detected, differing in the acylation pattern. One lipid A form was found to be decorated by four (*R*)-12:0 (3-OH) and one 12:0 acyl moieties, whereas the other was decorated by three (*R*)-12:0 (3-OH), one 10:0 and one 12:0.

From biological point of view, we demonstrated that the LPS and O-deacylated LPS from *C. littoralis* KMM 3880^T, *C. pacifica* KMM 3789^T and KMM 3878, *P. pacifica* KMM 9010^T and *P. sedimentorum* KMM 9023^T inhibit colony formation of different human cancer cell lines, including melanoma SK-MEL-5 and SK-MEL-28, colorectal carcinoma HT-29 and HCT-116 and breast adenocarcinoma MCF-7. We showed that sulfated OPS retain anticancer properties that open up new prospects for studying the antitumor activity of sulfated LPS and OPS from marine Gram-negative bacteria. In addition, the immunological studies demonstrated the very weak capability of marine Gram-negative bacteria LPS to elicit an immune response, as shown by the significantly lower release of pro-inflammatory cytokines in murine and human model systems compared to the potent immunostimulant *E. coli* LPS.

*Oral Presentation***A NOVEL ATYPICAL PKC-IOTA INHIBITOR, ECHINOCHROME A, ENHANCES CARDIOMYOCYTE DIFFERENTIATION FROM MOUSE EMBRYONIC STEM CELLS****Hyoung Kyu Kim^{1,*}, Sung Woo Cho^{1,*}, Natalia P. Mishchenko², Elena A. Vasileva², Sergey A. Fedoreyev², Valentin A. Stonik², and Jin Han¹**

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Echinochrome A (EchA) is a marine bioproduct extracted from sea urchins having antioxidant, antimicrobial, anti-inflammatory, and chelating effects which is the active component of the clinical drug histochrome. We investigated the potential use of Ech A for inducing cardiomyocyte differentiation from mouse embryonic stem cells (mESCs). We also assessed the effects of Ech A on mitochondrial mass, inner membrane potential ($\Delta\psi_m$), reactive oxygen species generation, and levels of Ca^{2+} . To identify the direct target of Ech A, we performed *in vitro* kinase activity and surface plasmon resonance binding assays. Ech A dose-dependently enhanced cardiomyocyte differentiation with higher beating rates. Ech A (50 μ M) increased the mitochondrial mass and membrane potential but did not alter the mitochondrial superoxide and Ca^{2+} levels. The *in vitro* kinase activity of the atypical protein kinase C-iota (PKC ι) was significantly decreased by 50 μ M of Ech A with an IC₅₀ for PKC ι activity of 107 μ M. Computational protein-ligand docking simulation results suggested the direct binding of Ech A to PKC ι , and surface plasmon resonance confirmed the direct binding with a low K_D of 6.3 nM. Therefore, Ech A is a potential drug for enhancing cardiomyocyte differentiation from mESCs through direct binding and inhibition of PKC ι activity.

The work was supported by grants from the Priority Research Centers Program, Basic Science Research Program, and International Research & Development Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science, and Technology (2010-0020224, 2015R1A2A1A13001900, 2015R1D1A1A01057937, and 2017K1A3A1A49070056). The study was carried out under support of the Ministry of Education and Science of the Russian Federation (RFMEFI61317X0076).

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*Oral Presentation***PHYSIOLOGICAL ROLE OF AMPK MODULATORY PROTEIN IN HEART AND MITOCHONDRIA****Jin Han***Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea*

Background and Purpose: Cereblon (CRBN) is a interacting protein with large-conductance calcium-activated potassium channels. A mutation of CRBN causes a mild type of mental retardation in humans. While, recent study suggested its novel function as AMPK inhibitor via direct interaction with AMPK $\alpha 1$ subunit. Disruption of CRBN gene enhanced hepatic AMPK activity and prevents high-fat diet induced obesity and insulin resistance in mice. The aim of study is to figure out the effect of CRBN KO in heart and its mitochondrial function.

Method and Results: Eight weeks of Control (CRBN^{+/+}) and CRBN KO (CRBN^{-/-}) models were examined their body weight, heart rate and heart/body ratio. In vivo cardiac functions of animals were assessed by echocardiography. To evaluate mitochondrial function of those animals, cardiac mitochondria of CRBN^{+/+} and CRBN^{-/-} were isolated then examined their ATP contents and ATP production rate, ROS production rate, oxygen consumption rate (OCR) and membrane potential ($\Delta\Psi_m$).

As results, the body weight, heart weight and heart/body ration were not significantly different between CRBN^{+/+} and CRBN^{-/-} mice. Echocardiography showed enhanced cardiac contractility in CRBN^{-/-} mice based on increased ejection fraction (%) and fractional shortening (%). CRBN KO enhance single cardiac myocyte cell contraction with intracellular Ca²⁺ transient. In their mitochondria, CRBN KO enhances Mitochondria Oxidative phosphorylation which lead to increase of basal ATP contents and substrate/ADP stimulated ATP production rate in CRBN^{-/-} mice than CRBN^{+/+}. In addition, basal H₂O₂ level and rotenone induced ROS production rates were significantly lower in CRBN^{-/-} mice than CRBN^{+/+}.

Conclusion: Our results suggested that CRBN is an important mitochondrial functional regulator which link cytosol to mitochondrial energy metabolism and Ca²⁺ signaling.

Keywords: Cereblon, cardiac contractility, mitochondrial energy metabolism. Ca²⁺ transient

*Oral Presentation***PROLONGED THREE DIMENSIONAL CULTURE OF PRIMARY HEPATOCYTES FORM DRUG METABOLISM ANALYSIS****Jong-Young Kwak***Department of Pharmacology, School of Medicine, Ajou University Suwon 19466, Korea*

Technical hurdles of primary hepatocyte culture are dedifferentiation, epithelial-mesenchymal transition, loss of function, and loss of proliferation. In this study, primary hepatocytes were cultured on poly(vinyl) alcohol (PVA) nanofibrous membrane with coculture of fibroblast in poly(caprolactone) nanofibrous scaffold. The hepatocytes adhered to PVA nanofiber membrane and formed spheroid upto 28 days. Cultured cells expressed E-cadherin and albumin during prolonged culture and maintained functions, including urea secretion and uptake of phenacetin. The functions of hepatocytes were enhanced by coculture of fibroblasts. The adherence of hepatocytes was increased using RGD-containing PVA nanofibers and cultured hepatocytes were grown in monolayer. Thus, our system can be used in drug metabolism analysis with culture primary hepatocytes.

*Oral Presentation***STEM CELL-BASED BASIC TO TRANSLATIONAL RESEARCH FOR IMMUNE DISORDERS: TOWARD THE ESTABLISHMENT OF CELLULAR PLATFORM FOR THE SCREENING OF MARINE NATURAL PRODUCTS****Hyung-Sik Kim**

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Mesenchymal stem cells (MSCs) are multipotent adult stromal cells that can self-renew and differentiate into various cell types of mesodermal lineage. Moreover, MSCs are recently known to possess regulatory function on immune cells which makes them a promising tool for the treatment of inflammatory and autoimmune diseases. The interaction between MSCs and immune cells through soluble factors and adhesion molecules has been reported to be crucial for the immunomodulatory effect of MSCs. However, MSC-based cell therapy still has potential limitations and the underlying mechanisms on specific disease remain largely unknown. The main purpose of these studies is to provide the better understanding of immune regulatory mechanisms focused on allergic immune responses and autoimmunity and to suggest the new insight available for bridging the current gap between scientific findings and clinical applications. Several murine models for immune disorders including inflammatory bowel disease (IBD), atopic dermatitis (AD) and rheumatoid arthritis (RA) were established and the efficacy of mesenchymal stem cells was determined. Administration of hMSCs reduced the severity of colitis, atopic dermatitis and arthritis in mice through the regulation of disease exacerbating immune cells. Therefore, these results might suggest novel therapeutic strategies for the treatment of allergic disorders and autoimmune diseases. Furthermore, the establishment of cellular or organoid platform using immune or stem cells can be promising and effective screening tool for marine natural products.

Keywords: mesenchymal stem cells, immunomodulation, inflammatory bowel disease, atopic dermatitis, rheumatoid arthritis

*Oral Presentation***UNIFIED AND PROTECTING-GROUP-FREE TOTAL SYNTHESIS OF NATURAL INDENE SESQUITERPENOIDS AND THEIR DERIVATIVES****Jeyun Jo, Myeonggyo Jeong, Sueun Jeon, Gibeom Kim, Hyunkee Lee, Hwayoung Yun****College of Pharmacy, Pusan National University, Busan 46241, Republic of Korea*

Natural indenenes (benzocyclopentadienes) are highly important carbocycles, which have shown various biological activities including antitumor, anti-hypercholesterolemic, antiallergic, anticonvulsant, herbicidal, fungicidal and antimicrobial activities. Anmindenols and nicotianasesterpenes were first isolated from a marine-derived bacterium *Streptomyces* sp. in 2014 and the leaves of *Nicotiana tabacum* in 2016, respectively. More recently, three new sesquiterpenoids were reported from the aerial parts of the *Polygonum barbatum*. These natural products are structurally unique indene sesquiterpenoids possessing a synthetically formidable exocyclic (E)-trisubstituted olefin.

Herein, we describe unified and protecting-group-free total synthesis of anmindenol A, nicotianasesterpenes A, B and a polygonum sesquiterpenoid. The key feature of our synthesis involves a stereoselective vinylogous Stork enamine aldol condensation, a Pd(0)-catalyzed regioselective hydrogenation, a substrate-controlled preparation of the key enamine precursor. Systematic studies on the influence of the size of alkyl aldehyde on the stereoselectivity of vinylogous Stork enamine aldol condensation have carried out. Considering the efficiency and synthetic feasibility of the synthetic route, our synthetic strategy seems widely applicable to structurally related indene derivatives.

*Oral Presentation***COULOMB NANORADIATOR-MEDIATED, SITE-SPECIFIC THROMBOLYTIC PROTON TREATMENT WITH FUCOIDAN-CONJUGATED MAGNETITE****Jae-Kun Jeon & Jong-Ki Kim***Catholic University of Daegu, School of Medicine**E-mail: jkkim@cu.ac.kr*

Traversing proton beam-irradiated, mid/high-Z nanoparticles produce site-specific enhancement of X-ray photon-electron emission via the Coulomb nanoradiator (CNR) effect, resulting in a nano- to micro-scale therapeutic effect at the nanoparticle-uptake target site. Here, we demonstrate the uptake of iron oxide nanoparticles (IONs) and nanoradiator-mediated, site-specific thrombolysis without damaging the vascular endothelium in an arterial thrombosis mouse model. The enhancement of low energy electron (LEE) emission and reactive oxygen species (ROS) production from traversing proton beam-irradiated IONs was examined. Flow recovery was only observed in CNR-treated mice, and greater than 50% removal of the thrombus was achieved. A 2.5-fold greater reduction in the thrombus-enabled flow recovery was observed in the CNR group compared with that observed in the untreated ION-only and proton-only control groups ($p < 0.01$).

Algal fucoidan is known to bind specifically with P-selectin from activated platelet. Prior study demonstrated fucoidan spontaneously exhibit thrombolysis by inhibiting plasma tPA-PAI1 binding and effectively enhancing free tPA. Therefore, Fucoidan-conjugated magnetite may have specific binding with activated platelet that was expressed in thrombosis initiation. We discuss various advantage of CNR thrombolysis with fucoidan-conjugated magnetite.

*Oral Presentation***ERYTHROPOIETIN (EPO) RECEPTOR EXPRESSION AND THE EFFECTS OF EPO ON DIFFUSE LARGE B CELL LYMPHOMAS****Jee-Yeong Jeong, Ph.D.**

Erythropoiesis-stimulating agents (ESAs), such as erythropoietin (EPO) and novel erythropoiesis stimulating protein (NESP), may alleviate anemia in diffuse large B-cell lymphoma (DLBCL) patients. However, many cancer cells express EPO receptors (EPOR), through which exogenously-administered ESAs potentially promote cancer cell growth. We conducted preclinical/phase II studies to investigate the safety and efficacy of ESAs for managing chemotherapy-related anemia in DLBCL patients. We examined EPOR expression in germinal center B-cell (GCB)- and activated B-cell (ABC)-DLBCL cell lines, and investigated the effects of ESAs on cell proliferation, and rituximab-mediated complement-dependent cytotoxicity (CDC). The clinical study enrolled 50 histologically-confirmed DLBCL patients receiving rituximab/cyclophosphamide/doxorubicin/vincristine/prednisolone (R-CHOP) who had hemoglobin levels <10.0 g/dL after a maximum of 3 R-CHOP cycles and received ≥ 4 doses of fixed-dose NESP (360 μg) once every 3 weeks. The primary endpoint was hematopoietic response (a hemoglobin increase of ≥ 2 g/dL from baseline or a hemoglobin increase to ≥ 12 g/dL). EPOR mRNA was detected in all GCB-DLBCL cell lines, but little/none was detected in ABC-DLBCL cell lines. GCB-DLBCL and ABC-DLBCL cell proliferation was unaffected by EPO or NESP. Rituximab-mediated CDC of DLBCL cell lines with/without EPOR expression was not affected adversely by EPO. In the clinical study, baseline mean hemoglobin was 9.19 g/dL; the overall mean change in hemoglobin was 1.59 ± 1.3 g/dL (16 weeks). Forty-eight percent of enrolled patients achieved a hematopoietic response. This study shows that ESAs do not affect the growth of DLBCL cells or rituximab-mediated CDC, and are effective and safe for DLBCL patients with anemia after R-CHOP.

*Oral Presentation***ANTIVIRAL ACTIVITY OF HISTOCHROME AND ANTIOXIDANT COMPOSITION CONTAINING ECHINOCHROME**

N.V. Krylova¹, S.A. Fedoreyev^{2*}, N.P. Mishchenko², E.A. Vasileva², V.F. Lavrov³, L.K. Ebraldidze³, G.N. Leonova¹

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The increase of viral infection's rate among all human infectious diseases up to 90% and the lack of vaccines and antiviral drugs is one of the most serious problems of modern health care. The currently used antiviral drugs usually have a rather low efficiency and a narrow spectrum of action (one drug against one virus) that determines the importance of the searching for non-toxic, effective, broad-spectrum antiviral drugs. The aim of this work was to study the activity of: a) HistoChrome[®] preparation, the active substance of which is echinochrome A (Ech A), a sea urchin pigment, and b) composition of antioxidants and Ech A against RNA and DNA viruses. Antioxidant drugs of HistoChrome[®] series were developed in PIBOC in 1998. HistoChrome[®] for intraocular injections (0.02 mg/ml of Ech A) is used for the treatment of inflammatory diseases of the retina, eye cornea, diabetic retinopathy and hemophthalmus of various origins. HistoChrome[®] for intravenous injections (10 mg/ml of Ech A) is used in cardiology for the treatment of acute myocardial infarction. Recently new applications of the drug product HistoChrome[®] for the treatment of hemorrhagic stroke and as a diuretic agent have been discovered.

HistoChrome[®] (10 mg/ml of Ech A), antioxidant composition based on Ech A (1 g of composition contains 0.30 g of Ech A, 0.30 g of ascorbic acid, 0.06 g of alpha-tocopherol acetate) and antioxidant composition without Ech A (1 g of placebo contains 0.30 g of ascorbic acid and 0.06 g of alpha-tocopherol) were used in this study.

Antiviral activity of the tested compounds was determined against RNA virus – tick-borne encephalitis virus (TBEV, Dal'negorsk strain), and DNA virus – herpes simplex virus type 1 (HSV-1, VR3 strain). Cytotoxicity and anti-TBEV/HSV-1 activity of compounds was investigated in PK cell culture (pig embryo kidney cells)/Vero cell culture (african green monkey kidney cells) subsequently. MTT assay revealed that 50% cytotoxic concentration (CC₅₀) of HistoChrome toward PK cells was 54.4±1.8 µg/ml, toward Vero cells – 60.5±3.1 µg/ml. The CC₅₀ value of the composition containing Ech A to PK cells was 20.3±0.9 µg/ml, to Vero cells - 23.1±2.2 µg/ml; the CC₅₀ value of the placebo composition without Ech A to PK cells was 521.7±5.3 µg/ml, to Vero cells - 530.9±9.4 µg/ml.

We investigated the effect of tested compounds on different stages of life cycle of TBEV and HSV-1: on the adsorption of the viruses to cells, on the early stage of the viral replication and on extracellular viral particles. The non-cytotoxic concentrations (10 µg/ml) of each compound and TBEV and HSV-1 in 10² TCID₅₀/ml were used in the assay. Antiviral activity of compounds was evaluated as inhibition rate (IR) of the virus-induced cytopathic effect (CPE) using MTT test. It was revealed that maximum inhibitory effect of tested compounds (88-96%) was observed after the pretreatment of RNA and DNA viruses with the compounds (virucidal

action). Inhibitory effect of compounds at the early stage of TBEV and HSV-1 replication (treatment of infected cells) was 31-39%, at the stage of viral adsorption (pretreatment of cells with compounds prior to infection) was 21-25%. To compare the antiviral activity of the tested compounds we investigated their direct virucidal effect on the model of TBEV infection (at different infectious doses of virus from 10^1 to 10^4 TCID₅₀/ml). After the pretreatment of each virus infectious dose with the compounds (at concentrations from 1 to 100 µg/ml), their 50% inhibitory concentration (IC₅₀) and selectivity index (SI) ($SI=CC_{50}/IC_{50}$) were calculated (Fig. 1). It was found that antiviral activity of antioxidant composition based on Ech A was higher than that of the HistoChrome® at all infectious doses of the TBEV ($p \leq 0.05$). At virus dose of 10^2 TCID₅₀/ml the direct virucidal effect of composition based on Ech A was characterized by $IC_{50}=12.6 \pm 1.5$ µg/ml and $SI=8.1$, whereas IC_{50} value of HistoChrome® amounted to 21.8 ± 2.6 µg/ml with $SI=2.5 \pm 0.2$, placebo composition without Ech A had $IC_{50} = 1304 \pm 145$ µg/ml and $SI = 0.4 \pm 0.1$. In addition, antioxidant composition based on Ech A much more effectively suppressed HSV-1 replication than HistoChrome® and placebo preparation ($p \leq 0.05$).

Thus, the study of the antiviral activity of HistoChrome® and of the composition of Ech A with natural antioxidants expands the range of their medical application and can serve as the basis for the development of antiviral drugs with a wide spectrum of action.

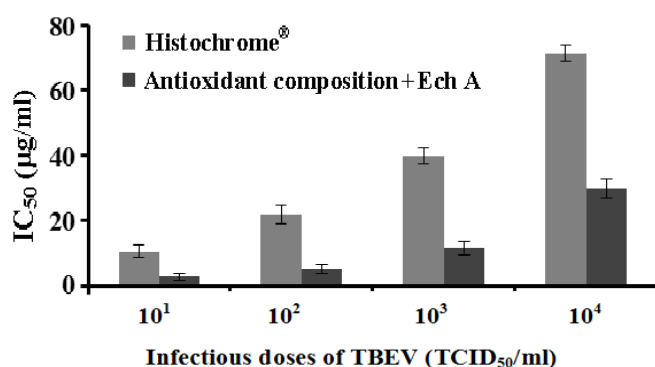


Fig. 1 50% inhibitory concentration of compounds (IC₅₀) at various infectious doses of the TBEV. Data are presented as mean ± SD (n = 4).

This study was supported by the grant of the Ministry of Education and Science of Russian Federation (project RFMEFI61317X0076).

*Oral Presentation***PIVOTAL CYTOPROTECTIVE MEDIATORS AND PROMISING THERAPEUTIC STRATEGIES FOR EPC-BASED CARDIOVASCULAR REGENERATION****Sang Hong Baek***Division of Cardiovascular Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea School of Medicine, Seoul, Republic of Korea; e-mail: whitesh@catholic.ac.kr*

Endothelial progenitor cell (EPC) would be one of potent therapeutic neovascular modulators in ischemic cardiovascular tissues. In response to ischemic injury signals, EPCs located in a bone marrow niche migrate to injury sites and form new vessels by secreting various vasculogenic factors including VEGF, SDF-1, and FGF, as well as by directly differentiating into endothelial cells. Nonetheless, most of engrafted EPCs do not survive under harsh ischemic conditions and nutrient depletion in ischemic tissues. Therefore, an understanding of diverse EPC-related cytoprotective mediators underlying EPC homeostasis in ischemic tissues may help to overcome current obstacles for EPC-mediated cell therapy. Additionally, to enhance EPC's functional capacity at ischemic sites, multiple strategies for cell survival should be considered, that is, preconditioning of EPCs with function-targeting drugs including natural compounds and hormones, virus mediated genetic modification, combined therapy with other stem/progenitor cells, and conglomeration with biomaterials.

Here, I will discuss multiple important cytoprotective mediators and promising therapeutic strategies for EPC-Based Cardiovascular Regeneration.

Oral Presentation

DEVELOPMENT OF NEW DRUG FORMS BASED ON ECHINOCHROME A USING ELECTROSPUN MICRO/NANOFIBERS

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The most well-known sea urchin pigment echinochrome A (Ech A) is the active substance in the antioxidant drug HistoChrome[®] produced in Russia from the sand dollar *Scaphechinus mirabilis* that is used in cardiology and ophthalmology. HistoChrome is available only in ampoules in the form of echinochrome di- and trisodium salts for intravenous injections or infusions, since Ech A is insoluble in water.

Recently appeared a large number of publications revealing a wide range of new pharmacological activities of Ech A, for example gastroprotective [1], anti-diabetic [2], anti-allergic [3], and mitochondria-protective properties against cardiotoxic drugs [4]. Therefore, development of new drug forms based on echinochrome with various components able to increase its solubility in water, to provide targeted and controlled release of the drug preserving or enhancing its pharmacological properties to expand the limits of drug's application is of great interest.

We obtained electrospun micro-/nanofibers from commercially used in pharmaceutical technology polymers (polyvinylpyrrolidone, polycaprolactone, cellulose acetate, hypromellose and polyethylene oxide) with Ech A incorporated and performed the *in vitro* dissolution tests at pH values 1.2 and 6.8. The most promising materials appeared to be polycaprolactone, hypromellose and polyethylene oxide since they provided a prolonged release of Ech A at pH 6.8.

This study was supported by the grant of the Ministry of Education and Science of Russian Federation (project RFMEFI61317X0076).

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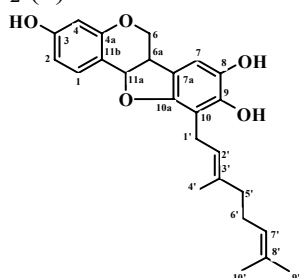
PRENYLATED POLYPHENOLIC COMPOUNDS FROM *LESPEDEZA BICOLOR*

Darya V. Tarbeeva, Sergey A. Fedoreyev, Marina V. Veselova

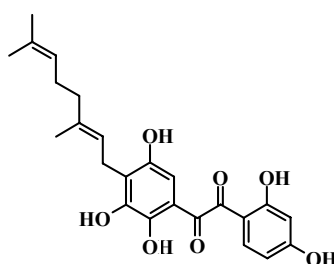
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Lespedeza bicolor is a legume shrub (Fabaceae) that is native to Japan and the South of the Russian Far East. *L. bicolor* has been used for the treatment of acute and chronic inflammation of urinary tract. Its extract is an active component of the medicine Lespephlan developed in the Russian Federation. This species is known to produce prenylated polyphenolic metabolites possessing antimicrobial, anti-inflammatory and antioxidant properties [1, 2]. We have studied the chemical composition and antioxidant activity of *L. bicolor* stem bark metabolites.

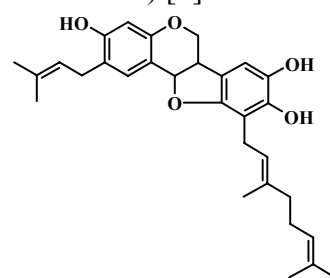
Five prenylated polyphenolic compounds have been found in the extract of *L. bicolor* stems. Compounds **1-4** have been isolated for the first time and their structures have been determined on the basis of NMR and CD spectral data as 6a,11a-dihydrolespedezol A₂ (**1**), 1-(2,4-dihydroxyphenyl)-2-(4-geranyl-2,3,5-trihydroxyphenyl)-etane-1,2-dione (bicoloketone) (**2**), 2-isoprenyllespedezol A₂ (**3**), 6a,11a-dihydrolespedezol A₃ (**4**) respectively. The molecular formulae of these compounds have been confirmed using HR-HPLC-MS technique. Lespedezol A₂ (**5**) had earlier been isolated from another *Lespedeza* species (*L. homoloba*) [3].



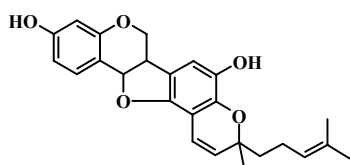
6a,11a-Dihydrolespedezol A₂ (**1**)



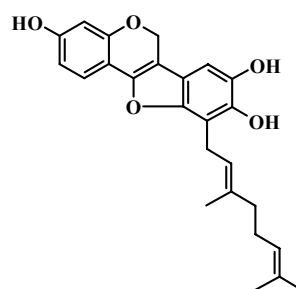
Bicoloketone (**2**)



2-Isoprenyllespedezol A₂ (**3**)



6a,11a-Dihydrolespedezol A₃ (**4**)



Lespedezol A₂ (**5**)

Several prenylated polyphenolic compounds with a methyl group attached to an aromatic ring had been isolated previously from *L. bicolor* root bark [2]. It should be noted that we have not found such compounds in *L. bicolor* stems. 6a,11a-Dihydrolespedezol A₃ (**4**) is a derivative of lespedezol A₃ isolated previously from *L. homoloba*.

We studied the DPPH scavenging effect of the isolated compounds as well as their “ferric reducing antioxidant power” (FRAP) directly determining the reducing capacity of a compound. The data on antioxidant activity of compounds **1-5** are shown in Table 1. We have found that

lespedezol A2 (**5**) previously isolated from *L. homoloba* possessed the most significant antiradical and antioxidant activity in both DPPH and FRAP tests. Compounds **1-4** possessed moderate antiradical activity and reducing capacity compared to reference antioxidant quercetin.

Table 1 – Antioxidant activity of polyphenolic compounds from *L. bicolor* stems

Compound	DPPH scavenging effect, IC ₅₀ (30 min), μM	FRAP assay, AAE*
Quercetin	9.17	1.52
Ascorbic acid	31.2	1.00
1	24.0	0.47
2	26.1	0.25
3	21.3	0.46
4	26.7	0.43
5	19.1	0.81

*The data of FRAP assay are given as ascorbic acid equivalents

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Poster communication

THE STRUCTURAL CHARACTERISTICS AND ANTICANCER ACTIVITY OF NATIVE AND MODIFIED FUCOIDANS FROM *SARGASSUM DUPLICATUM* AND *SARGASSUM FELDMANNII*

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Brown algae contain three types of biologically active polysaccharides. Alginic acids are the most studied and successfully used for a long time in the medical, pharmaceutical and cosmetic industries. Other valuable polysaccharides – laminarans and fucoidans, are less studied in comparison with alginic acids. Thus, their study is actual task today. Fucoidans are most interesting for researchers due to a wide spectrum of diverse biological activity, including anticancer, immunomodulating, antiviral and other [1]. Obtaining individual fractions of fucoidans, determination of the characteristics of their structure and biological activity are important for establishment of relationship «structure-activity», which is needed for development of medicines based on investigated polysaccharides.

The aim of this work was the investigation of the structural characteristics and anticancer activity *in vitro* of native and modified fucoidans from two brown algae of the genus *Sargassum*. Brown algae *S. duplicatum* and *S. feldmannii* were collected from Nhatrang bay (Socialist Republic of Vietnam) in June 2015. Samples of algae were defatted, dried and extracted by solution of diluted hydrochloric acid. Fucoidans were separated by anion-exchange chromatography and then purified from polyphenols to obtain fraction of individual polysaccharides.

From the data of the monosaccharide analysis, fucoidans were pure galactofucans with different ratios of fucose and galactose residues (51 and 49 mol % for fucoidan from *S. duplicatum* and 72 and 28 mol % for fucoidan from *S. feldmannii*, respectively. Obtained polysaccharides were sulfated (31.7% for fucoidan from *S. duplicatum* and 25.3% for fucoidan from *S. feldmannii*), fucoidan from *S. duplicatum* also was acetylated.

Non-carbohydrate substituents were removed from the fucoidans, and then the obtained fractions were investigated by 1D and 2D NMR spectroscopy. Main chain of modified galactofucan from *S. duplicatum* consisted of alternating 1,4-linked α -L-fucopyranose and β -D-galactopyranose residues with a small number of branches in the form of single α -L-fucopyranose [2]. The desulfated galactofucan from *S. feldmannii* contained monosaccharide residues of α -L-fucopyranose and β -D-galactopyranose linked by 1,3- and 1,4-bonds.

Soft agar method was selected for determination of the anticancer activity *in vitro* of native and modified fucoidans. The native fucoidans from *S. duplicatum* and *S. feldmannii* at the concentration of 200 µg/ml suppressed the colony formation of colon cancer cells DLD-1 on 70 and 57%, respectively. Deacetylated fucoidan from *S. duplicatum* displayed the similar activity with native fucoidan. But desulfation led to significant decreasing of anticancer activity. Thus, the presence of sulfate groups in investigated galactofucans influenced on their anticancer effect.

The work was supported by RFBR grant № 16-33-60023.

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Poster communication

STUDY OF THE ECHINOCHROME STABILITY AND THE PRODUCTS OF ITS OXIDATIVE TRANSFORMATION

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To ensure the quality of active pharmaceutical substances and finished drug products, impurities must be monitored carefully during process development, optimization, and process changeover. The isolation, characterization, and control of impurities in pharmaceutical substances are being reviewed with greater attention based on national regulatory and international guidelines.

Histochrome[®] is used in cardiological practice for the treatment of acute myocardial infarction and coronary heart diseases. Histochrome[®] is an effective medicine in ophthalmology and found a wide application in the treatment of corneal diseases for epithelialization acceleration, as well as retinal diseases such as a retinoprotector, at proliferative processes, of degenerations and different origin hemophthalmus. The active substance of the drug Histochrome[®] is echinochrome - 6-ethyl-2,3,5,6,7-pentahydroxynaphthaquinone (**1**). Due to the presence of a large number of phenolic hydroxyls, echinochrome is easily exposed to oxidative decomposition. In pharmaceuticals, the most common form of oxidative decomposition is auto-oxidation through a free-radical chain process.

Results from forced degradation studies of echinochrome in aqueous solution (pH 7.2) at room temperature we report here. A significant amount of the oxidation product (45-50%) was observed already 20 h after the beginning of the reaction. Products of the oxidation process **1** were identified using HPLC-DAD-MS (Shimadzu 2020).

Oxidation of **1** proceeded with the initial formation of dehydroechinochrome, the structure of which is established by NMR as 6-ethyl-2,3-dihydro-2,2,3,3,5,6,8-heptahydroxy-1,4-naphthoquinone (**2**) [1]. The high-resolution mass spectrum (ESI) of compound **2** shows a quasimolecular ion peak $[M-H]^-$ at m/z 299.0399 (calculated for $C_{12}H_{11}O_9$, 299.0409). The subsequent transformation of the unstable compound **2** with the elimination of CO_2 and H_2O and the cleavage of the C–C bond in the quinoid fragment gave ketoacids **3** and **4**. Carboxylic acids tend to lose carbon dioxide from the carboxyl groups during the isolation process. Therefore, to establish the structure of these compounds by NMR, stable methyl esters were obtained.

The relative retention times, absorption and mass spectra of compounds 2-4 were obtained. They will be used to develop a method for identifying impurities and determining the stability of substance and preparations based on echinochrome.

The study was supported by the program "Far East" (grant 18-4-037).

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Poster communication

CHITOSAN AND ITS ACYL DERIVATIVES INCLUDED IN LIPOSOMES PROTECT MICE AGAINST ENDOTOXIN SHOCK

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Endotoxins of gram-negative bacteria (lipopolysaccharide, LPS), penetrating into the bloodstream interact with immune factors and initiates the biosynthesis of effector molecules complex that cause endotoxic shock in large doses. An important problem of treating patients with endotoxemia is to find drugs to reduce the negative effects of endotoxin on the organism. The strategy of shock therapy is based on the removal of excessive amounts of LPS and/or blocking the binding of endotoxin to receptors on target cells [1]. An important way is the introduction of anti-endotoxin drugs into the human body and animals. Previously, we showed that natural polycations chitosans and its N-acylated derivatives form stable complexes with anionic LPS [2] and modulate significantly the biological activity of LPS [3]. Interactions of (LPS) with the polycation chitosan and its derivatives included in anionic liposomes were studied. The affinity of the interaction of LPS with a liposomal form of the N-acylated chitosan increases in comparison with, that are parent chitosan [4].

In this work we studied protective properties at endotoxemia of low molecular weight chitosan (Ch-LW, 4 -5 kD) and its N-monoacylated derivative (N-Ac-Ch-LW) in solution and in phospholipid liposomes when administrated intraperitoneally (i/p) and per-oral (p/o) to mice. Endotoxic shock was induced by injection i/p to the mice of LPS from *Escherichia coli* 055:B5 (7.5 mg/kg). Ch-LW was prepared by depolymerization of commercial chitosan with hydrogen peroxide. N-Ac-Ch-LW was obtained by acylated Ch-LW with 3-hydroxytetradecanoic acid hydroxysuccinimide ester. Liposomal forms of chitosans were obtained by entrapped of Ch-LW and N-Ac-Ch-LW on liposomes, preformed from lecithin, cholesterol and dicetyl phosphate [4]. It has been established that N-Ac-X-LM increases the stability of liposomes to the aggressive action of media simulating conditions in some sections of the gastrointestinal tract in 1.4-2 times. The interaction of Ch with LPS was shown to modulate significantly the biological activity of LPS. Using ELISA, it was shown that LPS in a complex with N-Ac-Ch-LW reduces induction of pro-inflammatory TNF- α in human blood by 40% in comparison with parent LPS. The formation of the LPS complex with N-Ac-Ch-LW does not affect the ability of endotoxin to stimulate blood cells to produce the pro-inflammatory cytokine IL-6. N-Ac-Ch-LW stimulated cellular immunity reactions. The introduction of hydrophobic substituent's into the molecule of chitosan increased its ability to stimulate the bactericidal activity, the adhesive properties and the absorption activity of neutrophils (at a concentration of 1-10 μ g/ml).

It was found that the double i/p administration of chitosan or its liposomal form when administered simultaneously with LPS did not significantly affect the survival rate of mice (Table). Intraperitoneal administration of Ac-Ch-LW 2 hours before the LPS injection better protected mice from the toxic effects of LPS, than its liposomal form. The maximum protective activity was shown by liposomes coated with Ac-Ch-LW at their ten-fold per-oral administration before induction of endotoxin shock with LPS. In this case Ac-Ch-LW showed weaker

protective properties from the effect of LPS (66% of surviving mice with an average lifetime of 38 h) compared with its liposomal form (83% of surviving mice at 51.4 h).

Thus, preventive ten-fold oral and double intraperitoneal injection of liposomal forms of chitosans reduces the toxic effect of endotoxin in the mice and significantly increases the survival rate (83-100%) of animals in the experiment. With oral administration of liposomal forms of chitosan, its protective effect may increase when bound to the epithelium of the gastrointestinal tract due to mucoadhesive properties of chitosans.

Table 1. Effect of X-HM, N-Ac-X-NM and their liposomal forms on the survival of BALB mice in endotoxin shock.

Group	Time of introduction	Preparations	Survival, %
I	1-fold i/p injection of LPS 7.5 mg/kg	Liposomes	20
II	2-fold i/p injection of N-Ac-Ch-LW 5 mg/kg	N-Ac-Ch-LW	100
III	2-fold i/p injection of 5 mg/kg, simultaneously with LPS	Ch-LW	27
		N-Ac-Ch-LW	25
		N-Ac-Ch-LW + liposomes	30
IV	2-fold i/p injection 5 mg/kg, 2 hours before the administration of LPS	Ch-LW	66
		N-Ac- Ch-LW	83
		Ch-LW+liposomes	90
		N-Ac-Ch-LW + liposomes	100
V	10-fold p/o administration 2.5 mg/kg, then LPS	Ch-LW	50
		N-Ac- Ch-LW	66
		Ch-LW + liposomes	76
		N-Ac- Ch-LW + liposomes	83

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Poster communication

STUDY OF SUBSTRATE SPECIFICITY OF TWO RECOMBINANT FUKOIDANASE FROM MARINE BACTERIA *WENYINGZHUANGIA FUCANILYTICA*

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Among polyanionic polysaccharides of brown algae possessing a wide spectrum of biological action (antiviral, antitumor, immunomodulatory, anti-inflammatory, anticoagulant, antiadhesive, anti-angiogenic), fucoidans are of the greatest interest for study [1]. In addition, fucoidans can be used for targeted drug delivery [2]. Fucoidans are sulfated homo- and heteropolysaccharides.

Enzymes that catalyze the cleavage of glycosidic bonds between sulfated fucose residues in fucoidan molecules are called fucoidanases. Interest in the study of fucoidanases, which catalyze certain transformations of fucoidans, is constantly increasing. This is due to the possibility of using them as tools for establishing the detailed structure of fucoidans. One of the most important properties of fucoidanases is specificity. The detailed specificity of the action of fucoidanases has not been sufficiently studied so far, it probably depends not only on the types of O-glycosidic bonds between fucose residues in sulfated polysaccharides, but also on the position of sulfate groups, like the specificity of carrageenases and heparinases [3, 4].

In this work, we studied the specificity of two recombinant fucoidanases from the marine bacterium *Wenylingzhuangia fucanilytica*. Genes of fucoidanases *fwf1* and *fwf2* were cloned in truncated forms without predicted N-terminal signal sequences and C-terminal sorting domain (secretion system). Genetic constructions coding fucoidanases were obtained by restriction free method. The recombinant fucoidanases FWF1 and FWF2 were produced in *Escherichia coli* strain Arctic Express.

To establish the substrate specificity of enzymes, information on the structure of the hydrolysis products of the substrate is usually used. We obtained products of enzymatic hydrolysis of fucoidan from *Fucus evanescens* by fucoidanases FWF1 and FWF2. The obtained oligosaccharides were analyzed by NMR spectroscopy using one- and two-dimensional techniques (¹H, ¹³C, COSY, TOCSY, HSQC, HMBC). Based on the analysis of the obtained spectra, was made a conclusion on the structure of the obtained oligosaccharides. The oligosaccharides obtained by the action of FWF1 fucoidanase differed in structure from the products of the action of fucoidanase FWF2, namely the degree of polymerization and the position of sulfate groups with fucose residues. The obtained oligosaccharides were used as

substrates to study of more detailed specificity of FWF1 and FWF2. The obtained data indicate that these enzymes have different substrate specificity.

Thus, the specificity of fucoidanases is characterized not only by the type of glycosidic bond cleaved by them, but also by the position of sulfate groups.

The work was supported by RFBR grant № 18-04-00905.

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EFFECT OF PENTACYCLIC GUANIDINE ALKALOIDS ON ACTIVITY OF NATURAL β -1,3-D-GLUCANASE FROM MARINE HYDROBIONTS**Yu.V. Dubrovskaya, T.N. Makarieva, L.K. Shubina, I.Yu. Bakunina***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

1,3- β -D-Glucanases are a large group of O-glycoside hydrolases that catalyze the hydrolysis of β -(1,3)-O-glycosidic bonds in β -(1,3)- and β -(1,3;1,6)-D-glucans (laminarans) and related polysaccharides. Glucanases are differed in the mode of action on the polymer substrate. There are endo- and exo-type 1,3- β -D-glucanases. Modification or blocking of these enzymes by selective inhibitors is probably the basis for regulation of their activity in the organism.

Marine sponges, which form an appreciable part of the biomass of marine macrofauna, contain substances that ensure successful ecological existence in different marine habitats, protect against the attack of predators and microbial pathogens, prevent overgrowth and help also in competition for space and nutrients. To date, a number of alkaloids of unique structures have been isolated from the marine Far-Eastern sponge *Monanchorapulchra*.

The effect of pentacyclic guanidine alkaloids monanchomycalin B, monanchocidin A and normonanchocidin A isolated from the Far-Eastern marine sponge *Monanchorapulchra* was investigated towards the activity of exo- β -1,3-D-glucanase from the filamentous marine fungus *Chaetomium indicum* and endo- β -1,3-D-glucanase LIV from the marine bivalve mollusk *Spisula sachalinensis*. All compounds were shown to be slow binding irreversible inhibitors of exo- β -1,3-D-glucanase and significantly activated endo- β -1,3-D-glucanase. The equilibrium inhibition constants (K_i , mM) and the inactivation rate constants (k_{inact} , min⁻¹) were determined for each compound. The inhibitory effect of alkaloids was shown to depend on the structure of the "anchor" part of the molecule of the compounds. Normonanchocidin A was the best inhibitor of exo- β -1,3-D-glucanase from fungi. In this study, exo-1,3- β -D-glucanase proved to be a sensitive model for detecting subtle differences in the mechanisms of action of these biologically highly active compounds, as well as for clarifying the role of individual structural elements in the biological action of the compound.

In sea sponges, various microorganisms are often found as symbionts. Probably, the studied compounds, by inhibiting such an important enzyme, perform defensive functions in the body of the sponge, protecting it from colonization by microorganisms including microscopic fungi. However the obtained results allow us to judge on the one of functions of these compounds in the complex ecosystem of the sponge and sponge's microbiome in the natural habitat.

The work was supported by the FEB RAS Grant No. 18-4-026.

Poster communication

MASS SPECTROMETRY OF SULFATED LAMINARAN DERIVATIVES, OBTAINED BY AUTOHYDROLYSIS IN HEAVY-OXYGEN WATER

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The mass spectrometry (MS) is a rapidly-developing, fast, sensitive and accurate instrumental method of molecular weight measurement. It is capable of measuring not only weights (MW) of molecular (parent) ions but also MW of their fragments (daughter ions) using a tandem (MS/MS) mode of operation. By the analysis of fragmentation pathways it is possible to establish structural features of the parent molecule. The structural analysis of native and modified carbohydrates is only small part of the application area of MS, but the important one, since the direct instrumental sequencing of carbohydrates is an unsolved problem. It is essentially to acquire information on fragmentation patterns of different samples of carbohydrates with known/defined structural features, especially if these samples are interesting due to their biological activities.

Recently, we have described a procedure modification of the biologically active complex sulfated polysaccharide – fucoidan. It was found that method of decomposition, an autohydrolysis (an autocatalysis of mild acid hydrolysis where own sulfate groups of the molecules act like the source of acid) was able to lower the molecular weight of fucoidan from *S. cichorioides* and to selectively remove the sulfate groups from C-2 positions of Fuc residues without loss of biological activity [1]. This method was further improved: we used heavy-oxygen water for autohydrolysis of known fucoidan samples for labeling the reducing end with ^{18}O [2]. Fragments were analyzed by electrospray ionization mass spectrometry (ESIMS/MS). The labeling improved our abilities for exact assignment of fragment ions: new fragmentation pathway was described: the substitution at C-4 generated $^{2,4}\text{A}$ -type ion (following nomenclature, suggested by Domon and Costello).

In present work we analyzed fragments of sulfated laminaran **DdLS** from brown alga *Dictyota dichotoma* during autohydrolysis in H_2^{18}O . Laminaran is 1,3;1,6-linked beta-D-glucan 1,3:1,6-linkage ratio 3:1, which was extracted, purified and sulfated with a pyridine solution of chlorosulfonic acid and dimethylformamide as described previously [3]. The MWs of **DdLS** was analyzed during autohydrolysis (60 °C) using high-performance size-exclusion chromatography: the Mw ranged from (intact) 12.5 kDa, 9.5 kDa at 24 h of autohydrolysis, 8.5 kDa at 48h and 6.4 kDa at 72 h. During the reaction the low MW fragments (1.2 Da - 2 kDa) were accumulated. ESIMS/MS analysis was capable of observing structural features of oligosaccharides only after additional mild acid hydrolysis in TFA (1N, H_2^{18}O) at 80 °C for 4 h. ESIMS/MS of disulfated glucose at m/z 169.99²⁻ revealed sulfation at C-2, C-4 and/or C-6. $^{0,3}\text{X}$ ion signal at 171.00 indicated sulfation at C-3. Probably, these residues were terminal or were single 1;6-branches [3]. ESIMS/MS of a trisulfated

glucobiose at m/z 193.66³⁻ was also informative: we observed [Glc-(1,3)-2,3,6-tri-OSO₃]³⁻, [Glc-2-OSO₃(1,6)-3,4-di-OSO₃]³⁻, [Glc-2,3-di-OSO₃(1,6)-3-OSO₃]³⁻ structural variants. Again, due to the labeling at the reducing end, we were able to distinguish between different fragment ions which could had same m/z without a label.

The estimation of MW of laminaran and its derivatives was performed in the center for collective use of scientific equipment "Far Eastern Center of Structural Studies". The study was supported by the Grant No. 16-13-10185 from the RSF (Russian Science Foundation).

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Poster communication

MODES OF ACTION OF SULFATED STEROIDS ON RECOMBINANT ENDO-1,3- β -D-GLUCANASE AND ALGINATE LYASE FROM MARINE BACTERIUM *FORMOSA ALGAE* KMM 3553

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There is lack of information about natural organic inhibitors of endo-1,3- β -D-glucanases (EC 3.2.1.39) and alginate lyases (EC 4.2.2.3) of marine origin. For endo-1,3- β -D-glucanases of marine mollusks, there was shown inhibition only by sulfated polyoxysteroids from marine sponges, starfishes and ophiuroids. Endo-1,3- β -D-glucanases of glycoside hydrolase family 16 (GH16) from *Pseudocardium sacchalinesis*, *Chlamys albidus* and *Patinopecten yessoensis* are irreversibly inhibited to 70% in the concentration of halistanol sulfate 1mkM (molecular ratio enzyme : substrate about 1:2.5).

There was studied action of halistanol sulfate, topsentiasterol sulfate D and chlorotopsentiasterol sulfate D from marine sponge *Halichondria* sp. on recombinant endo-1,3- β -D-glucanase GFA of GH16 family and alginate lyase ALFA3 of polysaccharide lyase PL7 family, both from marine bacterium *Formosa algae* KMM 3553.

In contrast to endo-1,3- β -D-glucanases of marine mollusks, the inhibiting action of sulfated steroids was relatively low. Halistanol sulfate caused 59% inhibition of GFA in molecular ratio 1:2665. The inhibition was reversible and competitive, constant of inhibition was 590 mkM. Topsentiasterol sulfate D appeared to be weaker agent and caused 38% inhibition of GFA in molecular ratio enzyme : substrate as 1:1262. Inhibition was reversible. Chlorotopsentiasterol sulfate D appeared to be the strongest one and caused 70% inhibition of GFA in ratio 1:2665 and 98% inhibition of GFA in ratio 1:2420, inhibition was irreversible. It's interesting to note that introducing of chlorine atom to the molecule changes the type of inhibition from reversible to irreversible.

Varying modes of action of substances with similar structure along with striking difference of action of halistanol sulfate on endo-1,3- β -D-glucanases of marine mollusks gives insight to the importance of surrounding of enzyme's active center with polypeptide chain fragments.

In case of alginate lyase (ALFA3) halistanol sulfate was the strongest agent and caused irreversible 100% inhibition in ratio 1:1530 and 59% inhibition in ratio 1:382. Topsentiasterol sulfate D was the weakest agent and caused reversible 59% inhibition in ratio 1:1455.

Chlorotopsentiasterol sulfate D caused 88% inhibition in ratio 1:2793 and irreversible 56% inhibition in ratio 1:348. Thus, it can be concluded that chlorotopsentiasterol sulfate D is the strongest inhibitor of GFA among tested substances, while halistanol sulfate is the strongest inhibitor of ALFA3.

This data supports the idea that inhibitors detect essential differences between enzymes, belonging to the same type of activity and even to the same structural family.

The work was supported by RFBR Grant № 18-04-00905 and RFBR Grant № 18-53-54002 V'iet_a.

INFLUENCE OF SAME MARINE FUNGI METABOLITES ON REACTIVE OXYGEN SPECIES LEVEL IN NEUROBLASTOMA CELLS**E.A. Pisl'yagin, E.S. Menchinskaya, O.F. Smetanina, E.A. Yurchenko***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Parkinson's disease is a chronic progressive disease of the brain, mainly associated with degeneration of dopaminergic neurons of a black substance and manifested by a combination of hypokinesia with rigidity, resting tremor and postural instability [1, 2]. Parkinson's disease (PD) is one of the most common neurodegenerative diseases of man, being the second after Alzheimer's disease, therefore PD is an extremely complex and socially significant problem of modern medicine [2].

Astaxanthin (which have marine origin) showed neuroprotective effect in *in vitro* models of Parkinson's disease, a significant inhibition of apoptosis and the formation of intracellular reactive oxygen species (ROS) in cells treated with 6-hydroxydopamine(6-OHDA) [3]. In this regard, a targeted search for marine substances with antioxidant activity to detect candidate compounds for *in vivo* studies is actual.

The aim of the work is to screen the metabolites of marine fungi *Aspergillus candidus* KMM 4676, *Eurotium niveoglaucum*, *Aspergillus flocculosus* and *Penicillium* sp. KMM 4672 for their antioxidant potential.

In our work, 38 metabolites of marine fungi were studied for radical-scavenger activity against DPPH and using the fluorescent dichlorofluorescein diacetate probe, the effect of lead compounds on the formation of reactive oxygen species (ROS) under the action of 6-hydroxydopamine in mouse neuroblastoma Neuro2a cells was evaluated. The DPPH-radical scavenging activity was shown by new terphenylline B ($IC_{50}=73.1 \mu M$). Also known polyketides tetrahydroauroglaucin, flavoglaucin, neohinulin and cryptoehinulin D (as racemate) was shown significant radical-scavenger activity ($IC_{50}=55.5 \mu M$, $58.1 \mu M$, $62.6 \mu M$, $58.0 \mu M$, respectively). Seven compounds showed a significant inhibition of ROS formation in 6-OHDA-treated Neuro 2A cells. It can be noted that, despite different approaches to the study of ROS level, terphenylline B and tetrahydroauroglaucin showed high activity in both tests. Isochromene [4] inhibited the ROS formation in Neuro2a cells by 46%, while in the DPPH test, it was only 38%. Mactanamide [5] had no DPPH-radical scavenging activity but it was shown decreasing of ROS level in 6-OHDA-treated Neuro2a cells to control value.

Thus, our data suggest the regulating effect of the same marine fungal metabolites on the level of active forms of oxygen and the redox status of neuronal cells.

CYTOTOXICITY OF SOME MARINE FUNGI METABOLITES AGAINST CANCER CELLS

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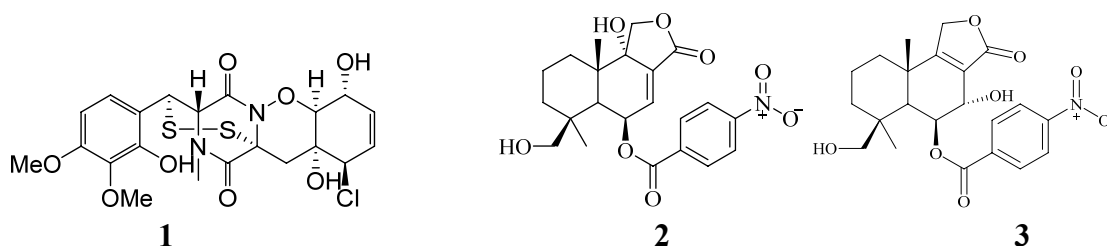
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The aim of this study was investigate the cytotoxic activity of some low-molecular secondary metabolites of marine fungi-micromycetes. The influence of 38 compounds on the viability of mice neuroblastoma cells Neuro 2a, as well as human breast cancer cells MCF-7 and prostate cancer 22Rv1, was studied by MTT test.

It was found that neochinulines B and C from the Vietnamese fungi *Eurotium niveoglaucum* demonstrated a cytotoxic activity against mice Neuro2a cells. Their EC_{50} concentrations were 50.9 μ M and 40.6 μ M, respectively. 4"-Dehydroxycandidusine A and candidusine A [1] were less toxic $EC_{50} = 78.9 \mu$ M and 75.7 μ M, respectively.

N-methylpretrichodermamide B **1** at a concentration of 100 μ M induced the death of 47% of the neuroblastoma cells. It was found earlier, that N-methylpretrichodermamide B was highly cytotoxic against 22Rv1, PC-3, and LNCaP cancer cells with IC_{50} 0.51, 5.11, and 1.76 μ M, respectively [2].

The highest cytotoxic activity was demonstrated by sesquiterpenoid nitrobenzoyl esters 9 α ,14-dihydroxy-6 β -p-nitrobenzoylcinnamolide **2** with $EC_{50} = 4.9 \mu$ M, while its analogue **3** did not affect to viability of cells. Treatment human breast cancer cells (MCF-7) with compound **2** shown a less cytotoxic effect ($EC_{50} = 59.6 \mu$ M), compared to mice neuroblastoma cells, whereas compound **3** is practically inactive. The effect of compound **1** on drug-resistant prostate cancer cells 22Rv1 was very significant with $EC_{50} = 3 \mu$ M. Compound **2** was no toxic against this cancer cells also.



Thus, the same marine fungal metabolites have big potential as anticancer compounds.

The work was supported by RFBR grant № 18-34-00737.

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POLYPHENOLIC COMPOUNDS FROM *AMPELOPSIS JAPONICA* INHIBIT WNT SIGNALING

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Triple negative breast cancer (TNBC) is the most aggressive breast cancer type. The majority of TNBC cases are associated with improper activation of the intracellular Wnt signaling pathway. Targeted hormonal and anti-HER₂ therapies is ineffective when treating TNBC. Thus, the search for selective modulators of the Wnt pathway, including metabolites from natural sources, is one of the most important tasks in the field of pharmacological research aimed at treating cancer.

One of the plants producing metabolites that can inhibit Wnt signaling is a Far Eastern endemic plant *Ampelopsis japonica*. Its water-alcohol extract has been shown to selectively suppress Wnt activity in TNBC cells. In order to isolate the active compounds we applied this extract to a polyamide column and subsequently eluted polyphenolic compounds with hexane (fraction A), chlorophorm (fraction B), chlorophorm:etanol 10:1 (fraction C), ethanol (fraction D) and water (fraction E). All the fractions were tested for the ability to effectively suppress Wnt signaling in HTB-19-TOPFLASH cells using the double luciferase analysis technique. Fractions C and D showed the most significant activity. Treatment of cells by this fractions has reduced the Wnt-activated luminescence to the background level. The ethanol fraction was then subsequently applied to a Toyaperl HW-50 column and to a C-18 column. Fractions 4, 11 and 14 significantly inhibited Wnt signaling (Fig. 1) at a concentration of 5 ng per 1 mkl of culture medium. According to LC-ESI-MS data these fractions contained individual compounds **1**, **2** and **3** respectively. Their molecular masses and maxima in UV spectra are shown in Table 1.

Table 1 – LC-MS data for compounds **1-3**.

Compound	UV-spectrum	[M+H] ⁺	[M-H] ⁻
1	280	291	289
2	274	-	331
3	274	-	331

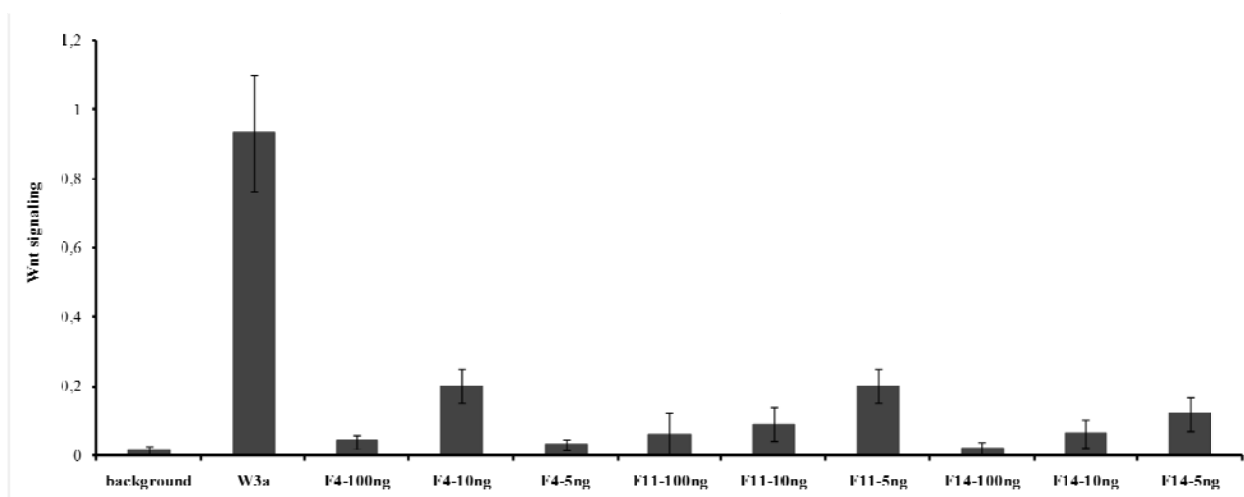


Fig. 1 – Wnt suppressing activity of polyphenolic compounds from *Ampelopsis japonica* (background – background luciferase activity, W3a – the activation of luminescence by Wnt3a protein).

This study was supported by the Russian Foundation for Basic Research (grant 18-34-00502 mol_a).

Poster communication

**THE FUCOIDANS FROM BROWN ALGAE *SACCHARINA CICHORIOIDES*,
SACCHARINA JAPONICA AND *LAMINARIA LONGIPES***

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The obtaining of medicines based on natural substances is one of the important tasks of modern pharmaceutical industry. The polysaccharides of brown algae are non-toxic and have wide spectrum of biological activity. Fucoidans – sulfated polysaccharides of the cell-wall matrix of brown algae, have different biological effects such as anticoagulant, thrombolytic, immunomodulating, antitumor and antiviral. These polysaccharides have the various structures: they divided into pure α -L-fucans and heteropolysaccharides, which can contain the residues of galactose, mannose, xylose, uronic acids and other monosaccharides. The investigation of fucoidans is relevant for medicine, agriculture, cosmetic and food industries [1].

Brown algae of order Laminariales are widespread on the Far East of Russia. We have studied structural characteristics of fucoidans from three kinds of brown algae: *Saccharina cichorioides*, *S. japonica* (Terpeniya Bay, Sea of Japan, 2017) and *Laminaria longipes* (Shikotan Island, Sea of Japan, 1996) belonged to Order Laminariales.

The individual fractions of fucoidans were obtained using the defatting of algae by 70% aqueous ethanol, extraction of dry algal residues by solution of HCl, and anion exchange chromatography of polysaccharide extracts. In the result we isolated the one fucoidan fraction from *S. cichorioides*, two fractions from *S. japonica*, and one fraction from *L. longipes* with yields 4.1, 0.5, 0.9, and 0.35% of dry algal weight, respectively.

The structural characteristics of fucoidans, such as monosaccharide composition, sulfate content, the presence of protein and polyphenol impurities, were determined. Also all polysaccharides were investigated by the NMR spectroscopy. It was shown that the first fraction of fucoidan from *S. japonica* was sulfated (21%) and acetylated galactofucan with trace amounts of mannose and xylose; the second fraction – sulfated (30%) and acetylated polysaccharide, containing mainly fucose residues. Fucoidans from *S. cichorioides* and *L. longipes* were sulfated (32 and 36%, respectively) fucans. Polysaccharide from *S. cichorioides* also contained a trace amount of galactose residues. The protein and polyphenol impurities were absent in the all fucoidan fractions.

Thus, the brown algae *S. cichorioides*, *S. japonica* and *L. longipes* are the accessible source of fucoidans consisted predominantly from fucose, the further study of the structure and activity of which are very interesting.

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Poster communication

ALKALINE PHOSPHATASE / PHOSPHODIESTERASE FROM MARINE BACTERIUM *COBETIA AMPHILECTI* KMM 296

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Alkaline phosphatases are widely distributed in nature and play a key role in the utilization of soluble phosphorus by hydrolytic cleavage of phosphate monoesters under alkaline conditions, releasing inorganic phosphate from many phosphate-containing compounds [1]. At present, three families of prokaryotic alkaline phosphatases (PhoA, PhoD and PhoX) are known. They differ in structure, substrate specificity, and dependent on different metal ions to exhibit their activity. It has been shown that phosphatase PhoD, belonging to the phosphatase/phosphodiesterase family, is more common in marine bacteria than PhoA and PhoX phosphatases that suggests its important role in marine microorganisms [2].

Using the specific primers based on the gene sequence encoding PhoD-like protein, which was found in the full-length genome of the marine bacterium *Cobetia amphilecti* KMM 296 (GenBank, no. JQJA00000000.1) [3], the recombinant salt-resistant metal-dependent phosphatase/phosphodiesterase CamPhoD with a specific activity 1.6 U/mg (0.025 M, pH 9.0, 15 mM p-NNP) was produced in the *Escherichia coli* cells DH5 α , and its physical and chemical properties were studied. The molecular weight of the subunit of the dimeric CamPhoD was 55 kDa. The enzyme was activated by Co²⁺, Mg²⁺ and Fe³⁺ in a concentration of 2 mM and exhibited its maximal activity at pH 9.2. The Zn²⁺, Cu²⁺, Mn²⁺ ions, as well as EDTA and EGTA, did not significantly affect the activity of CamPhoD. The study of CamPhoD specificity revealed that the enzyme catalyze the phosphorus cleavage in the following range: TTP \geq dGMP \geq UTP \geq pNPP \geq CDP \geq TMP \geq bis-pNPP \geq 5'-pNP-TMP. The optimal temperature for the exhibition of CamPhoD activity was 45 °C, while it was completely inhibited at 65 °C. The salts NaCl and KCl did not affect the activity of CamPhoD at concentrations up to 1 M, whereas an incubation mixture containing 1.5 M NaCl and KCl reduced the enzyme activity by 50% and 80%, respectively.

Thus, the marine bacterium *Cobetia amphilecti* KMM 296 gene has been confirmed to encode the functionally active extracellular phosphatase/phosphodiesterase of the PhoD family. For the first time the recombinant CamPhoD was obtained to explore its enzymatic properties.

The work was supported by the program "Far East" grant № 18-4-051.

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Poster communication

THE EFFECT OF PH ON THE CHAPERONE ACTIVITY OF SKP FROM *YERSINIA PSEUDOTUBERCULOSIS*

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Chaperones perform different functions: they can help fold and inhibit aggregation of the unfolded protein; accompany the newly synthesized protein to the site of its localization in the cell, supporting in the unfolded, active for translocation state; prevent lethal nonspecific association of proteins under stress conditions for the cells. Incorrect protein folding in the cell and/or aggregation thereof is the cause of destructive human diseases, such as prion infections, Alzheimer's disease, and type 2 diabetes. In this regard, the study of the properties and mechanisms of chaperone functioning represents not only fundamental, but also of practical interest, associated with the search for approaches and the creation of drugs for the prevention and treatment of neurodegenerative diseases and to enhance the body's resistance to stress. One of the most important periplasmic chaperones of gram-negative bacteria is Skp protein. Along with the ability to interact with the outer membrane proteins as a chaperone, Skp proteins have other properties that can be biologically and physiologically significant. They exhibit lipopolysaccharide- and DNA-binding activity [1-4] and are chemoattractants for monocytes and polymorphonuclear leukocytes [5]. Previously, we showed that Skp *Yersinia pseudotuberculosis* bound human and rabbit IgG by a non-immune manner (bypassing the antigen-binding sites of IgG (antibodies)) both in the form monomer (Skp) [6] and in the form homotrimer (Skp₃) [7]. The aim of this study was to evaluate the effect of pH on the behavior of Skp *Y. pseudotuberculosis* in solution, to determine the qualitative and quantitative characteristics of its chaperone- and immunoglobulin-binding activities. Protein Skp was expressed in *E. coli*, and was isolated from the cells and purified as described previously [7]. Commercial samples of Fc- and Fab-fragments of human IgG were used as protein substrates for binding to Skp chaperone. According to dynamic light scattering (DLS), recombinant Skp (rSkp) in sodium acetate buffer, pH 5.0 had has exhibits a monomodal particle size distribution with a hydrodynamic radius (R_H) of 3.6 ± 0.3 nm. When the pH of the medium changes from acidic to alkaline, rSkp aggregation is observed, and its rate increases as pH approaches the isoelectric point of the protein ($pI = 9.33$). Within 6 days after isolation, rSkp in the buffer, pH 5.0 had a Z-average hydrodynamic radius (Z-average) of only 6.4 nm. Already in the first 30 minutes after the transfer of the

chaperone to solutions with neutral and alkaline pH values of 6.7 and 7.9, its Z-average sharply increased to 61 and 155 nm, respectively, and by 24 hours was 341 and 383 nm, respectively. Fc- and Fab-fragments of human IgG ($M_r = 52$ kDa) in PBS showed a monomodal particle size distribution with $R_H = 3.3 \pm 0.3$ nm, the Z-average values of the samples were 14 and 11 nm, respectively. In buffer solutions with a lower ionic strength and pH values of 5.1, 6.7 and 7.9, these proteins showed an increase in Z-average, distribution width (multimodal distribution), and relative content (%) of particles with R_H greater than 10 nm. Such behavior indicates self-association and subsequent aggregation of Fc- and Fab-fragments of human IgG under the test conditions. In the presence of rSkp, the rate of these processes decreased significantly: an increase in the relative content (%) of particles with R_H greater than 10 nm and the Z-average of the samples was slowed down. The experiments carried out by us showed a significant influence of the pH of the medium on the rSkp chaperone activity. At acidic pH of the solution (under the conditions of which rSkp is most stable) the chaperone formed complexes with Fc and Fab that retained the stability for up to 20 hours and had R_H to 10 nm. In solutions with neutral and alkaline pH values (6.7 and 7.9), the rSkp chaperone activity decreased markedly. The low molecular weight Skp-Fc, -Fab complexes (R_H up to 10 nm), which dominated in the solution for the first 30 minutes (98.2-99.8%), were replaced in the course of time by coarse particles with R_H from 10 to above 200 nm: at pH 6.7 to 24 hours in the solution the particles larger than 200 nm predominated (63.3 and 100% by volume for Skp-Fc and Skp -Fab, respectively), and at pH 7.9 only particles larger than 200 nm were present.

Chaperone activity of Skp in respect of Fc- and Fab fragments was different. At pH 7.9, rSkp bound Fc to form small size complexes (R_H to 10 nm) that were stable for 4 hours, whereas the interaction of the chaperone with Fab under these conditions resulted in the formation of particles with R_H in the range of 10-200 nm. This may be the result of the differences between Fc and Fab in their binding strength to rSkp. Using biosensor analysis based on the plasmon resonance method, we have shown that K_A rSkp with the Fc fragment is greater than with the Fab fragment in 1.4 and 2.2 times at pH 7.0 ($9.7 \times 10^3 M^{-1}$ and $6.9 \times 10^3 M^{-1}$) and at pH 8.0 ($2.0 \times 10^6 M^{-1}$ and $9.1 \times 10^5 M^{-1}$), respectively.

In recent years, more information has appeared in the literature that the structural transformations of proteins in cells caused by changes in the pH of the medium increase or decrease the activity of chaperone proteins [8-10]. The results obtained in this work also demonstrate that the chaperone activity of Skp *Y. pseudotuberculosis* is pH dependent. Recombinant Skp stably binds Fc- and Fab-fragments of human IgG at low pH, thereby preventing their irreversible aggregation, but when neutralizing the pH of the medium, chaperone forms weakly stable complexes with them, which do not exclude further self-association and aggregation of protein substrates.

This work was partially supported by a grant from the RFBR № 16-08-00679.

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Poster communication

THE CULTURE TEMPERATURE AFFECTS THE PROPERTIES OF THE *YERSINIA PSEUDOTUBERCULOSIS* PORIN INCLUSION BODIES AND THE STRUCTURE OF THE RECOMBINANT PORIN

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The recombinant proteins are widely used in various fields of industry (pharmaceutical, food, cosmetic), in scientific research and diagnostics, so interest in their production is constantly increasing. One of the most widely used bacterial systems expressing heterologous proteins remains *E. coli*. However, the production of recombinant proteins in *E. coli* is often accompanied by the formation of so-called inclusion bodies (IBs), which are aggregates of an almost pure and potentially ready-to-use target protein. In this connection, the task is to obtain IBs containing as much as possible correctly folded functionally and biologically active protein. The solution of this problem requires, among other things, a deep knowledge of the structure and mechanisms of the formation of IBs. The proportion of the functionally active protein in IBs is characteristic of the target protein sequence, but also depends on many other factors such as a combination of the parameters of the expression process, including the cell growth temperature. A number of studies have shown that a decrease in the culture temperature effectively improves the quality of the protein in IBs [1, 2].

The purpose of this study is to determine the effect of cell cultivation temperature on the properties and structure of IB. The OmpF porin *Yersinia pseudotuberculosis* was expressed in *E. coli* at 37 (IB-37), 30 (IB-30) and 18 °C (IB-18) in the form of IBs, which were isolated, purified and characterized. The stability of inclusion bodies in aqueous solutions of urea (1-8 M) and SDS (0.02-0.1%) and the state of the recombinant protein in these solutions (particle size, degree of polydispersity, secondary structure) were investigated. Solubilization of IBs in urea or SDS was monitored by measuring the turbidity at 350 nm, the size and size distribution of the particles of the recombinant porin in the solutions were measured by dynamic light scattering (DLS).

All studied IBs were progressively more soluble with increasing urea concentration from 1 to 8 M. The turbidity of TB solutions decreases sharply with an increase in the urea concentration from 3 to 5 M and remains almost unchanged in 7-8 M urea. The time of incubation of IBs in urea in the interval 1 - 24 hours does not visibly affect their solubilization.

Dissolution of TB-30 and TB-18 mainly occurred at lower urea concentrations: from 2 to 4 M and from 2 to 3 M when incubated in solution for 1 and 24 hours, respectively. At the same time, IB-18 in comparison with IB-30, are more soluble in urea, and the incubation time is a more significant factor for them affecting solubilization. Thus, IBs expressed at a lower temperature were less stable in urea. According to the DLS data, in the IBs solutions in urea there are two populations of particles. One of them is represented by large particles (200-500 nm), which, apparently, are intact IBs and particles close to them in size, the other - consists of relatively small (<100 nm) particles that appear as a result of solubilization of IBs, and is very polydisperse. In solutions with a lower concentration of urea (1-4 M), the large particles are mostly present, while a population of the small particles with R_H 40-80 nm and 40-60 nm predominates (up to 70% of the total number of particles) in more concentrated solutions of 5-8 M and 4-6 M for IB-37 and IB-18-30, respectively. It should be noted that the large protein particles (secondary aggregates) accumulate in the concentrated urea solution, and their number increases with increasing incubation time. As is known, the fully unfolded OmpF porin has a hydrodynamic radius $R_H = 8.2 \pm 0.3$ nm measured by DLS [3], hence the recombinant porin in urea solutions is present in the form of oligomers (associates) and aggregates, but not monomers. IBs dissolve well in dilute solutions of SDS: the turbidity of IBs suspensions drops sharply with an increase in detergent concentration from 0.02 to 0.06 (0.08)%. An increase in IBs exposure in the detergent leads to an increase in the amount of dissolved recombinant protein, while the concentration range of the detergent in which IBs solubilization occurs remains the same. The most effective IBs dissolve in the first hours of contact with SDS. Analysis of solutions of TB in the detergent by DLS showed that the particle size decreases with increasing detergent concentration from 0.02 to 0.1% and the incubation time from 2 to 24 hours. The particles obtained by solubilizing IB-37 in 0.06-0.1% SDS for 24 hours had an average R_H of about 40 nm. The smallest particles, R_H 11-20 nm, were found in 0.08% SDS solutions. At the same time, the solution of TB-18 in 0.06% SDS incubated for 24 hours contained particles with R_H of 5.4-7.1 nm, the proportion of which was 86.9% and 81% of the total number and volume of particles, respectively. In IBs solutions with a detergent concentration of 0.08-0.2%, smaller particles (R_H 3.7-4.6 nm) are present, whose content is about 99% both in number and volume, and they have mainly monomodal size distribution. Thus, in a detergent solution, the recombinant porin from TB-18 is in monomeric form, unlike that of TB-37, which under these conditions exists as an oligomer.

The structure of rOmpF porin solubilized from TB and the effect of urea and detergent on it have been studied using CD-spectroscopy in the far UV region. Analysis of CD spectra allowed us to conclude that recombinant porin in IBs, expressed at 37, 30, and 18 °C, had a pronounced secondary structure with predominance of β -structure. However, differences in the

type of β -structure between TB synthesized at 37 °C and at lower temperatures are likely to occur. This assumption is supported by the CD spectra of recombinant porin in urea, which have negative maxima at 230 and 210-220 nm and positive bands at 202 and 198-199 nm for IB-37 and IB-18, 30, respectively. The denaturants that are used to solubilize IBs cause changes in the conformation of the porin. The resistance of the secondary structure of recombinant porin to SDS and urea decreases with a decrease in the temperature of its expression. The porins from IB-37 and IB-18 have the greatest and the least structural stability, respectively. The process of protein denaturation occurs in two stages: the accumulation of the α -helix by decreasing the β -structure (the ratio $\theta_{217-216} / \theta_{209-207}$ for the protein in the solution is 0.04, 0.06 and 0.08% SDS decreases) and the subsequent increase in the content disordered structure (the short-wavelength of the CD spectrum minimum shifts from 207-209 to 204-203 nm, and the band intensity increases). In the case of rOmpF from IB-37, an increase in the concentration of denaturants and incubation time in solution is not accompanied by a marked increase in the content of the disordered structure.

Thus, a decrease in the expression temperature is accompanied by an increase in the solubility of IBs in SDS and urea, an increase in the content of the native-like β -structure in the porin, but leads to a decrease in the stability of its structure to the denaturants. The novel information obtained in the study contributes to the understanding of the structural organization and mechanisms of formation of IBs and other protein aggregates and to the development of approaches for the production of biologically active IBs.

This work was partially by supported by a grant from RFBR № 16-08-00679.

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Poster communication

APET_x-LIKE PEPTIDE INTERACTION WITH ASIC_s CHANNELS: COMPARATIVE IN SILICO STUDY

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At present, one of the main problems of bioorganic chemistry is the investigation of molecular mechanisms of cell receptors functional activity, in particular the proton-gated ion channels (ASICs) which are expressed mainly by neurons of the central (ASIC1, ASIC2) and peripheral (ASIC3) nervous system. ASIC channels are involved in performing of the sensory neurons basic functions, such as mechanical sensitivity, perception of pain caused by inflammation and local infection. ASICs are also implicated in neurodegenerative diseases (ischemia, Parkinson's disease) [5]. However, there are relatively few ASICs modulators of peptide nature [3, 4, 6, 9, 11] compared to low molecular weight.

Previously, π -AnmTX Hcr 1b-1 (Hcr 1b-1), peptide (41 aa, 4537 Da), was obtained from the sea anemone *Heteractis crispa* by a multistage liquid chromatography. It was elicited that Hcr 1b-1 induced an inhibition of human hASIC3 peak current with an IC₅₀ of 5.5 μ M [11]. The BLAST search algorithm revealed that Hcr 1b 1 belongs to the group of APET_x2-like toxins, among which only APET_x2 from the sea anemone *Anthopleura elegantissima* possesses the inhibitory effect on ASIC3 currents (IC₅₀ ~ 175 and ~ 63 nM for human hASIC3 and rat rASIC3, respectively) [6]. APET_x2 structure was used as a prototype for creating of Hcr 1b-1 spatial model (49% of identity) [4, 9].

The experimentally determined ASIC3 3D structure is not available yet, the channel homology model of structure was generated by program MODELLER 9.11 [8]. Currently, the spatial structures of the extracellular and transmembrane domains have been determined only for chicken cASIC1 mutants, both in the free and bound state with psalmotoxin-1 PcTx1 (IC₅₀ ~ 0.9 nM for rASIC1a) from the venom of spider *Psalmopoeus cambridgei* [1, 2, 7]. The cASIC1 structure extracted from the latter complex (4FZ0) was used as a template for hASIC3 structural model generating (54% of identity). The results of molecular docking performed by ClusPro 2.0 [10] and following refinement with ToxDock servers [12] and molecular dynamics simulations of generated Hcr 1b-1–1hASIC3 complex demonstrated that hASIC3 and cASIC1 share the peptide binding sites (Fig. 1a). These sites are located between neighboring channel subunits overlapping the entrance to deep pocket with proton-binding site.

The analysis of intermolecular interactions in Hcr 1b-1–hASIC3 complex revealed the importance for binding of the peptide residues Lys5, Asp40, Arg41 (Fig. 1b) which are involved in hydrogen bonds and salt bridges formation with channel Asp163, Arg226, Asp227 ones, respectively. Interestingly, Arg226 is characteristic for ASIC3 channels, but not for ASIC1 ones with Gly at equivalent position. It should be noted that a highly basic flexible loop containing Arg26, Arg27, Arg28 residues (Fig. 1b) is responsible for the complex PcTx1–cASIC1 formation [1]. Unlike PcTx1, Hcr 1b-1 peptide is characterized by localization of basic residues His7, Lys5, and Arg41 on β -strands which leads to decreasing of both flexibility and ability to bury into deep pocket and to reach the proton-binding site (Fig. 1b). This may explain the higher IC50 value of Hcr 1b-1 for inhibition of hASIC3 compared to one of PcTx1 for inhibition of ASIC1. The charge inversion caused by replacement of cASIC1 Glu354Arg residue which occupies hASIC3 equal position, prevents electrostatic interactions with Arg27 of PcTx1. Moreover, in this case the important for receptor recognition by peptide π σ interactions of Arg27 with Phe174 are lost. Our molecular modeling results revealed that there are several variable residues among ASIC3 and ASIC1 at the area of peptide binding site. According to the literature data, they play a crucial role for PcTx1 binding to cASIC1 [1, 2, 7]. We suggest that Hcr 1b-1 and PcTx1 complementarity to these hASIC3 and cASIC1 variable residues is rather responsible for the binding specificity, than the difference in structure packing of peptide.

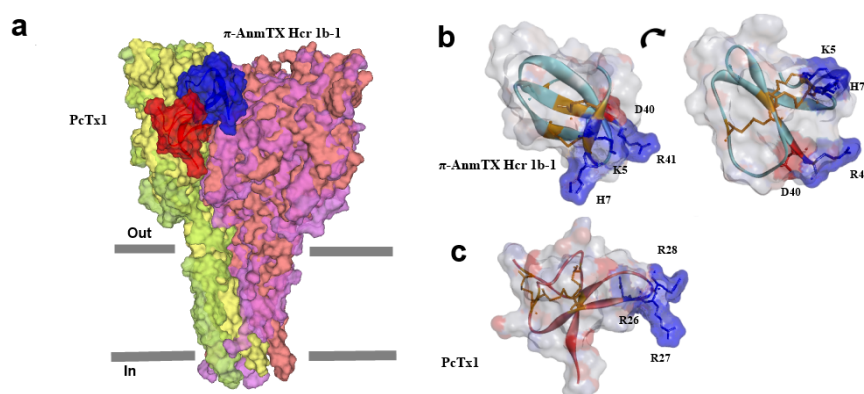


Figure 1. The structure diagram of Hcr 1b-1–hASIC3 and PcTx1–cASIC1 complexes. (a) Superposition of Hcr 1b-1--hASIC3 complex structural model and X-ray structure of the PcTx1–cASIC1 (PDB ID 4FZO [9]). Each subunit of hASIC3 (green, violet) and cASIC1 (yellow, cerise) is shown as solvent-accessible surface. Hcr 1b-1 (blue), PcTx1 (red) are shown as ribbon diagram and transparent solvent-accessible surface. (b, c) Hcr 1b-1 and PcTx1 [9] are shown as ribbon diagram with transparent solvent-accessible surface colored in accordance with the charge. The disulfide bridges stabilizing peptide structures presented as orange sticks. The side chains of residues important for peptides interaction with the channels, namely Lys5, His7, Arg41 (blue), and Asp40 (red) of Hcr 1b-1 and Arg26, Arg27, Arg28 (blue) of PcTx1 are presented as sticks.

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FIRST STEPS IN STUDYING OF JELLYFISH GONIONEMUS VERTENS VENOM

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Jellyfish venoms are one of the most challenging tasks for toxinologists and have considerable difficulty in studying, which explains the almost complete non-study of venoms of some species that have an obvious threat to human health.

Jellyfish *Gonionemus vertens* is not lethal to human health, but the effects of envenomation cause severe, painful, in some cases, long-term symptoms, which makes it the most dangerous kind of jellyfish of the Sea of Japan. The venom of this jellyfish has not been studied before, and information about the compounds responsible for its biological activity is absent. This work is devoted to the isolation and identification of these compounds, as well as to identify the types of biological activities responsible for the symptoms of poisoning.

In the course of this work, *G. vertens* venom was separated by gel filtration chromatography to seven fractions for biological activity investigation. Some fractions showed activities against the rat TRPV1, mouse TRPV2, human TRPV3 receptors expressed in CHO cells in a Fluo-4–based intracellular calcium assay. One fraction showed high toxic activity on coastal crabs and decreased viability of mouse neuroblastoma Neuro2a cells. This fraction demonstrated 80% inhibition of labeled alpha-bungarotoxin binding to muscle-type *Torpedo californica* ray and human $\alpha 7$ nAChRs. Also this fraction included components showed 11% inhibition of rat ASIC1a channel expressed in *Xenopus laevis* oocytes.

The results of the work demonstrate the activities of substances of the *G. vertens* venom in various tests, as well as their preservation after purification and separation processes and their thermal stability, which makes this species an intriguing and promising object for toxinological and proteomic studies.

This work was supported by the Far East Program (grant No. 18-4-009), RFBR grant 18-04-01366 (I.K., radioligand assay) and the Russian Science Foundation (grant No. 16-15-00167) (Y.K. and I.M., calcium assays on TRP channels).

Poster communication

EXPRESSION OF GENES OF PIGMENT DIFFERENTIATION THROUGHOUT THE DEVELOPMENT AND IN CULTURED EMBRYONIC CELLS OF THE SAND DOLLAR *SCAPHECHINUS MIRABILIS*

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Sea urchins, producing secondary metabolites, such as naphthoquinone pigments are possible sources of valuable biologically active substances for the pharmaceutical industry. Two gene families involved in the induction of pigment differentiation in the sand dollar *S. mirabilis* were selected for analysis, and their expression level was evaluated by quantitative real-time-PCR (q-RT-PCR) throughout the development and in cell cultures. *In vivo*, the highest level of expression of the *pks* and *sult* genes in sand dollar embryos was observed at the blastula and gastrula stages. In unfertilized eggs and spermatozooids, only trace amounts of these transcripts were detected. *In vitro*, genes of interest were also expressed significantly in blastula-derived cell cultures, confirming that primary embryonic cell cultures are suitable models for *in vitro* investigation of pigment differentiation. Pigmentation has been shown to be more intense when the cells were cultured in sea urchin coelomic fluids rather than in seawater. The maximal number of pigment cells of *S. mirabilis* was detected in the coelomic fluid of injured sea urchins. This assay is a useful tool for assessing the production of naphthoquinone pigments during development and cultivation and is important for the development of new techniques in marine biotechnology and pharmacology.

ENZYMES, PROTEINS, AND SOLUBLE MULTI-PROTEIN COMPLEX OF EGGS OF SEA URCHIN *STRONGYLOCENTROTUS INTERMEDIUS***Georgy A. Nevinsky¹, Svetlana E. Soboleva¹, Natalia I. Menzorova², Pavel S. Dmitrenok²**¹*SB RAS Institute of Chemical Biology and Fundamental Medicine, 8 Lavrentiev Ave., Novosibirsk 630090, Russia*²*G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Eggs of the sea urchin are an exceptionally convenient model for studying the patterns of development from embryo to the body.

Genome of all living beings exists in a dynamic equilibrium between ongoing DNA damage and reversal of the damage, a process known as DNA repair. Decrease in DNA repair capacity ultimately manifests itself in the form of mutagenesis, carcinogenesis, or cell death, and is implicated in a number of human diseases. DNA repair is crucial both for rapidly proliferating cells, in which lesions in DNA interfere with replication fork progress and may be converted into mutations upon replication, and for terminally differentiated cells, which sometimes have to maintain their genome integrity for the entire lifespan of the organism and have cell division-dependent checkpoints downregulated or turned off. Several pathways have been defined in most organisms, including direct reversal, base excision repair (BER), nucleotide excision repair, mismatch repair, non-homologous end-joining, and recombination repair. Of those, BER, which removes small non-bulky lesions, the most abundant type of spontaneous and induced DNA lesions, seems to be of the greatest importance in multicellular animals, judging from the embryonic lethality of knockouts inactivating the whole pathway. In the course of BER, one of several enzymes belonging to the class of DNA glycosylases excises a damaged base from DNA, leaving an apurinic/apyrimidinic (AP) site; then an AP endonuclease cleaves DNA at the AP site providing a free 3'-OH terminus, which is further used by a DNA polymerase to incorporate a normal dNMP. Finally, DNA ligase restores the integrity of the formerly damaged strand.

In actively proliferating cells, such as cells of the developing embryo, DNA repair is crucial for preventing accumulation of mutations and synchronizing cell division. Sea urchin embryo growth was analyzed and extracts were prepared. The relative activity of DNA polymerase, apurinic/apyrimidinic (AP) endonuclease, uracil-DNA glycosylase, 8-oxoguanine-DNA glycosylase, and other glycosylases were analyzed using specific oligonucleotide substrates of these enzymes; the reaction products were resolved by denaturing 20% polyacrylamide gel electrophoresis [1]. We have characterized the profile of several key base excision repair activities in the developing embryos (2 blastomers to mid-pluteus) of the grey sea urchin, *Strongylocentrotus intermedius*. The uracil-DNA glycosylase specific activity sharply increased after blastula hatching, whereas the specific activity of 8-oxoguanine-DNA glycosylase steadily decreased over the course of the development. The AP-endonuclease activity gradually increased but dropped at the last sampled stage (mid-pluteus 2). The DNA polymerase activity was high at the first cleavage divisions and then quickly decreased, showing a transient peak at blastula hatching. It seems that the developing sea urchin embryo encounters different DNA-damaging factors early in development within the protective envelope and later as a free-floating larva, with hatching necessitating adaptation to the shift in genotoxic stress conditions. No correlation was observed between the dynamics of the enzyme activities and published gene expression data

from developing congeneric species, *S. purpuratus*. The results suggest that base excision repair enzymes may be regulated in the sea urchin embryos at the level of covalent modification or protein stability.

It was proposed that most biological processes are performed by different protein complexes. In contrast to individual proteins and enzymes, their complexes usually have other biological functions, and their formation may be important system process for the expansion of diversity and biological functions of different molecules. Identification and characterization of embryonic components including proteins and their multi-protein complexes seem to be very important for an understanding of embryo function. We have isolated and analyzed for the first time a very stable multi-protein complex (SPC, ~1100 kDa) from the soluble fraction of extracts of the sea urchin embryos [2]. By FPLC gel filtration the SPC was well separated from other extract proteins. SPC is stable in different drastic conditions but dissociates moderately in the presence of 8 M urea+1.0 M NaCl. According to SDS-PAGE data, this complex contains many major, moderate and minor proteins with molecular masses (MMs) from 10 to 95 kDa. The SPC was destroyed by 8 M urea or SDS, and its components were separated using thin layer chromatography (TLC), ion-exchange chromatography, gel filtration, and reverse phase chromatography (RPC). Using MALDI mass spectrometry of partially dissociated SPC, it was shown, that the complex contains not only proteins (10-95 kDa), but also few dozens of peptides with MMs from 2 to 9.5 kDa. Short peptides form very strong complexes, which at the treatment of SPC with urea or SDS can be partially break down into smaller complexes having different peptide compositions. RPC of these complexes after all type of above-mentioned chromatographies led to detection from 6 to 11 distinct peaks corresponding to new complexes containing up to a few dozens of peptides. The SPCs possess alkaline phosphatase activity. Progress in the study of embryos protein complexes can help to understand their biological functions.

The work was supported by Russian Foundation for Basic Research (RFBR) grant 18-04-00442.

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3. This research was made partially possible by grants from Russian Foundation for Basic Research (16-04-00609) to Dmitrenok PS.

Poster communication

STEROID HORMONE BIOSYNTHESIS MEETS BIOTECHNOLOGY

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Six cytochromes P450 are involved in steroid hormone biosynthesis. We investigated the regulation of crucial steps by protein-protein interactions as well as steroid hormone intermediates. Special attention is given to glucocorticoid and mineralocorticoid formation catalyzed by CYPs of the CYP11B subfamily. Molecular genetic and biochemical analyses of patients with defects in these CYPs were performed. Since CYP11B2 and CYP11B1 are targets for the development of drugs against hypertension, congestive heart failure and metabolic syndrome, systems for testing new potential drugs had to be developed. We were able to establish reliable test systems using stable cell cultures, yeast expression and purified proteins. In addition, to better understand structure-function relationships of steroid hydroxylases, corresponding bacterial enzymes have been cloned and analyzed. The structural basis for the regio- und stereo-selectivity of hydroxylation by human and bacterial steroid hydroxylases was characterized using computer modeling, protein design and directed evolution of steroid hydroxylases. Furthermore, protein crystallization revealed the structural basis for the stereo- and regio-selectivity of hydroxylation of steroid molecules. Finally, by analyzing CYPs for their potential to hydroxylate steroids and using the possibilities of protein design and evolution, important results for the application of these enzymes in biotechnology for the sustainable production of drugs were obtained.

Poster communication

POLYSACCHARIDE FROM *PSEUDOPTEROGORGIA AMERICANA* MODULATES IMMUNE RESPONSE IN MACROPHAGES

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The marine polysaccharides have a profound impact on the regulation of immune response. However, the mechanism of immune modulation by polysaccharides isolated from the coral *Pseudopterogorgia americana* (PPA) in mammalian cells remains unclear. Here, we demonstrated that PPA activated macrophages through toll-like receptor 4. PPA induced the expression of TNF- α , IL-6 and COX-2 in mouse macrophages, but had no effect on the expression of induce NO and iNOS. The PPA-mediated macrophages activation was regulated by ROS, MAPK, PKC and NF- κ B. Notably, PPA pretreatment resulted in a reduced expression of TNF- α and IL-6 in LPS-activated macrophages through the downregulation of IRAK2 expression, MAPK phosphorylation and NF- κ B activation, indicating that PPA induced LPS tolerance in macrophages. Taken together, our data indicate that PPA has the potential to be used as an immune modulator in mammals.

Poster communication

MECHANISMS OF PRENATAL EFFECTS OF CAFFEINE IN RATS: ROLE OF NITRIC OXIDE

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Caffeine is the most widely consumed psychoactive compound worldwide. It is mostly found in coffee, tea, energizing drinks and in some drugs. In recent years, caffeinated energy drinks are increasingly popular among adolescents. The number of women, who use psychotropic substances during pregnancy, including caffeine, also increases from year to year. It was showed that the prenatal effect of psychostimulants could lead to a delay in development and behavioral disorders in their offspring [3]. However, mechanisms by which caffeine could influence the brain development are still not well established. It was found that ability to bind to adenosine receptors of the brain plays a key role in the neurochemical mechanisms of the stimulating effect of caffeine [2]. At the same time, its possible interaction with other neurotransmitter systems has not been studied enough. Results of our group and other have led to hypothesis that neurophysiological effects of psychostimulant drugs are mediated by free radicals [1]. The aim of the work was to investigate a role of neuronal messenger nitric oxide (NO) in the neurophysiological prenatal effects of caffeine.

Experiments were performed using male Wistar rats, originating from pregnant female rats. Pregnant dams received solution of caffeine (1g/L) or water as the sole source of fluid during all their gestation period. NO content in brain structures was determined using the direct quantitative method of electron paramagnetic resonance. No major difference in physiological characteristics of juvenile male rats admitted prenatally by caffeine or water was observed. In the first four postnatal days NO generation in the brain of rats of both studied groups was significantly reduced in comparison with the values of NO in the brain of adult rats. However, NO content was lower in the brain of juvenile male rats received caffeine on postnatal day 2 and 3 but not 4, as compared with control animals. It was found that prolonged intake of caffeine by female rats during pregnancy led to increase in locomotor activity, as well as hypoalgesia in their offspring at the age of 1 month. Preliminary administration of NO synthase inhibitor induced analgesic and anxiolytic effects in rats treated prenatally, both caffeine and water. It was shown that rats from mothers who received caffeine during pregnancy found the underwater platform in the water maze faster than control group. This fact might indicate that the spatial memory of the experimental group is better developing than in the control group of animals. It was found that the administration of inhibitor of NO-synthase before the first training session in the water maze

significantly increased the latency period in both studied groups of animals. Although, it should be noted that rats treated with NO-synthase inhibitors and caffeine reached the platform more quickly than those received prenatally water. It was found that NO content in rat hippocampus was significantly increased in the group of rats receiving prenatal caffeine compared to the control rats before testing in the water-maze. Preliminary administration of NO-synthase inhibitor has led to significant decrease in NO generation in the hippocampus of rat treated by caffeine and water prenatally. In summary, we can conclude that the nitrergic system of the brain is involved in prenatal effects of caffeine in rats.

The work is supported by RFBR grant 16-04-00722.

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LIPOSOMES AS CARRIER FOR ECHINOCHROME**V.I. Gorbach, V.N. Davydova, V.P. Glazunov, I.M. Yermak***G.B. Elyakov Pacific Institute of Bioorganic Chemistry FEB RAS, Vladivostok, Russia*

Natural substances possess unique characteristics and abundantly available in nature. The most abundant sea urchin pigment echinochrome A (6-ethyl-2,3,5,7,8-pentahydroxy-1,4-naphthoquinone) (Ech) exhibits a wide range of pharmacological activities [2, 6]. Ech is soluble in organic solvents, but its solubility in water is poor that limits its application. Early we have shown that inclusion of Ech in complexes with CRGs decreased its oxidative degradation and improved its solubility [8]. The liposomal form of Ech can be the best formulation to improve the bioavailability of Ech in cells. Liposomes are vesicles consisting of an aqueous core enclosed by one or several lipid bilayers and can encapsulate a wide range of drugs, both hydrophilic and hydrophobic, either in the aqueous core or in the lipid membrane [4]. The function of the liposomes strongly depends on their properties, especially their size and charge. Liposomes can be coated by charged polymer with mucoadhesive properties [7]. The coating of the liposomes with a mucoadhesive polymer as carrageenan can improve the stability of the liposomes [4, 7].

The present study is primarily focused on the preparation and characterizations of conventional liposomes and liposomes containing Ech/CRG composition.

CRG was isolated from a sterile form of red algae (*Chondrus armatus*) with an average molecular weight of 560 kDa determined by viscosimetry. The substance Echinochrome (registration number in the Russian Federation is P N002362/01 [STATE REGISTER OF DRUGS (as of December 5, 2016) Part 2]) was obtained in G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Vladivostok, as powder.

Conventional and CRG/Ech containing liposomes were produced using standard thin film hydration and sonication method. An extruder with membranes 0.1 μm and 0.4 μm was used to obtain liposomes homogeneous in sizes. Liposomes produced with larger pore membranes (liposomes-0.4) yielded a polydispersity suspension with a mean hydrodynamic diameter 430.3 ± 29.8 nm. Unilamellar liposomal suspensions with a low polydispersity were prepared with membrane having a pore size of $= 0.1$ μm (liposomes-0.1). These liposomes were homogeneous particles and had monomodal distribution with a mean diameter 125.6 ± 2.5 nm.

The stability of liposomes during 24 hours in solution was studied by dynamic light scattering (DLS). Liposomes-0.1 were more stable than liposomes-0.4. They preserve their stability after 24 h storing in water as also in saline solution. Aggregation and increase in polydispersity index (from 0.108 to 0.315) were observed for liposomes-0.4 after 24 h storing in water. Raising hydrodynamic diameter from 430.3 ± 29.8 nm to 616.3 ± 45.7 nm was also registered.

It is known that liposomes are advantageous in encapsulating different drugs. Hydrophilic drugs are entrapped in the aqueous layer, while hydrophobic drugs are stuck in the lipid bilayers [1]. CRG was used as a soluble matrix. The liposomes loaded water solutions of CRG/Ech were prepared using standard thin lipid film method followed by sonication. The concentration of Ech included in liposomes was determined after its disruption with treatment of butanol/water mixture. The entrapment efficiency of Ech in butanol layer was determined spectrophotometrically at 468 nm. It was 46 % that may be due to its hydrophobic nature and stuck in the lipid bilayers, as was shown for hydrophobic drug [6]. Water-soluble drugs have lower

encapsulation in the liposomes compared to their lipophilic counterparts [3]. Indeed, according to our data, only 8% of carrageenan was included in the liposomes.

To evaluate of the stability of Ech in the liposomes the absorption at 468 nm were determined. Two characteristic absorption bands of the native Ech at 339 nm and 468 nm were observed in the spectrum of Ech included in liposomes. It is known that oxidation form Ech has absorption band at 390 nm [5]. We did not registered such a band in obtained spectrum of Ech from 300 to 650 nm. So the Ech did not transformed after lyophilization of liposomes and their storage.

Loading the liposomes with negatively charged polymers CRG resulted in reversal of the ζ -potential to negative values, which together with increase in size propose the formation of liposomes coating with polymer. ζ -potential of liposomes CRG/EchA-0.1 and CRG/EchA-0.4 were -24.4 and -15.6 mV respectively. A higher value of negative charge for liposomes-0.1 in comparison with liposomes-0.4, is probably due to a greater amount of CRG that covers of smaller liposomes.

The present study reported on the properties of liposomes with and without CRG/Ech encapsulation. The possibility of using liposomes as a suitable carrier system for different applications depends on their properties. Particle size and ζ -potential are important properties that determine stability of liposomes. Value of the ζ -potential is also useful tool in controlling the aggregation, fusion and precipitation of liposomes.

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Poster communication

APPLICATION OF NATURAL POLYHYDROXYNAPHTHOQUINONE ECHINOCHROME A FOR TREATMENT AND PREVENTION OF ATHEROSCLEROSIS

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In conditions of clinical trial, the effect of low doses of natural polyhydroxynaphthoquinone pigment of sea urchin *Scaphechinus mirabilis* (sand dollar) – echinochrome A was studied as part of HistoChrome® medicinal preparation and Thymarin biologically active food additive (dietary supplement), on lipid metabolism, antioxidant status and condition of immune system including cytokine profile, in patients with cardiovascular system diseases. The results of clinical studies on the effects of low doses (0.4 – 2.5 mg/day) of polyhydroxylated 1,4-naphthoquinones on patients with cardiovascular pathology demonstrate correction of lipid and carbohydrate metabolism disorders, as well as functional changes in the immune system in these patients. After treatment with HistoChrome, as can be seen from the investigation /experimental data, there is a transition of the immune system to quite different level of functioning, wherein energy supply of the with ATP is normalized through activation of peroxisomal-mitochondrial biogenesis and increase in the number of mitochondria in the cells. The increase in HLA-DR expression reflects the strengthening of the capability of immune cells of intercellular cooperation and increases the efficiency of antigen processing and presentation, and the decrease in the expression level of a marker of apoptosis CD95+ is a testament to the completion of the processes of cell renewal in the body.

EchA producing hydrogen peroxide in the body increases the expression of PGC-1 α co-activator of the receptors of PPARS family (H₂O₂ is the cause of over expression of PGC-1 α [1]), which functioning increases the number of peroxisomes in the cells. The increase in the number of peroxisomes leads to the increase in the content of catalase in these organelles. The decrease in the level of H₂O₂ in the cells exposed to catalase expressed in peroxisomes is the cause of lowering the values of CD95+ after therapy of patients with HistoChrome preparation. Hydrogen peroxide is capable of inducing the expression of Fas-ligand (CD178+) in predominantly activated cells of the immune system such as Th1 and natural killers (NK) [3]. The simultaneous increase in the levels of CD95+ and CD178+, apparently contributes to

apoptosis of the cells in the body that is confirmed in our study by increased levels of creatine kinase MB – intracellular marker of the decay of cardiomyocytes in the blood of patients after treatment with EchA.

The observed changes in cytokine profile in patients after their treatment with HistoChrome preparation indicate directed action of EchA to normalize the population of macrophages, shifting their differentiation to phase M2, thereby reducing the development of atherosclerotic inflammation. Anti-atherosclerotic effect of HistoChrome is most likely associated with the activation by EchA of receptors-activators of peroxisome proliferation and nuclear factor Nrf2, the functioning of which is aimed at the urgent expression of enzymes detoxifying compounds of quinonoid nature, such as DT-diaphorase. In addition to detoxifying functions, this enzyme plays an important protective role in cardiovascular diseases [2]. EchA is the agonist of receptors of PPARs family and, in addition to inhibiting of LDL oxidation and transferring of the immune system to a higher level of functioning and normalizing OXPHOS in it, it eliminates a great numbers of atherosclerotic inflammation.

The data obtained allow recommending HistoChrome and Thymarin dietary supplements as a means of complementary therapy for patients with cardiovascular diseases to correct disorders of metabolic processes, as well as for preventive monotherapy among these patients during remission to prolong and stabilize it.

Keywords: sea urchin, polyhydroxynaphthoquinone, atherosclerosis.

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Poster communication

NEW FUCOIDAN FRACTIONS FROM BROWN ALGA *FUCUS EVANESCENS*: STRUCTURE AND BIOLOGICAL ACTIVITY

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The classical extraction method of sulphated fucose-containing polysaccharides – fucoidans is a time-consuming process. Different irreversible structure changes in native polysaccharides can occur during this. The purpose of our investigation was to research the possibility of an ultrasound-assisted extraction method for isolation and separation of polysaccharides of a brown alga *F. evanescens*, and to study the structures and biological activities of the new fractions of fucoidans.

The application of an ultrasound-assisted extraction method, combined with ion exchange chromatography, has allowed separation of the total fucoidan fraction (F1) from the brown alga *Fucus evanescens* into two fractions, F2 and F3, in the ratio 1:0.2. The traditionally extracted F1 differs from F3 only by the acetate groups at C3 being somewhat predominant. F2 is described as having acetate groups located chaotically, and contains galactose and xylose residues, in addition to fucose. F3 consists of fucose residues acetylated almost exclusively at C3. Therefore, the new polysaccharide extracted from preprocessed alga *F. evanescens* by ultrasound is shown to be a fucan built from a repeating disaccharide chain, where the fucose residues are connected by (1→3) and (1→4) glycoside links, sulphated at C2 and acetylated at C3 of (1 → 4)-linked α -L-fucose residues.

New fucoidan showed anticancer activity *in vitro* towards human colon carcinoma cells. Its *in vitro* anticancer activity towards human colon cancer cells was shown to be comparable to the fraction obtained by the classical method. The study of the biological activity of the new fucoidan will be continued. the fraction obtained by the classical method. The study of the biological activity of the new fucoidan will be continued.

Keywords: seaweed, extraction, sulfated polysaccharides, fucoidan, activity.

Poster communication

DESIGN IN BIOTECHNOLOGY: CAN LIVING ORGANISMS IN BIOASSAYS BE REPLACED ON ENZYMES?

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Historically, the application of bioluminescence in toxicology began with the usage of luminous bacteria and they are still widely used. As opposed to other test objects such as paramecia, algae, and so on, the luminous bacteria assay is faster (< 30 min). However, as with other organisms, luminous bacteria is petulant. The failure to maintain the stable state of bacterial culture during measurements and storage results in low accuracy of measurement, a clear disadvantage of this method caused by the “petulance”. The bacteria react to the toxic substances either by decreasing or by increasing the luminous intensity, often leading to ambiguous interpretation of results. Because of these shortcomings the luminous bacteria assay didn't show reliable results. The new approach to develop the bioluminescent enzymatic biosensors, toxicity bioassays and reagents has been described. To solve the problem of how to detect, identify, and measure the contents of the numerous chemical compounds in environmental monitoring, food product monitoring, and medical diagnostics, the bioluminescent enzymatic toxicity assays were proposed, wherein the NAD(P)H:FMN-oxidoreductase +luciferase substitutes for living organisms. The immobilized reagent Enzymolum was introduced to facilitate and accelerate the development of cost-competitive enzymatic systems for use in biosensors. Prototype biosensors offer cost advantages, versatility, high sensitivity, rapid response, extended shelf and flexible storage conditions. Due to the coupling with luciferase, it is possible to design new enzymatic bioassays in toxicology and combine them into a set. The set includes key enzymes of metabolic processes such as LDH, trypsin and others. The set of bioluminescent enzymatic toxicity assays was used for monitoring natural and laboratory aquatic ecosystems, soil contamination, as well as for toxicity analysis of pesticides and sanitary assessment of nanomaterials. The new possibilities of enzymatic bioassays are discussed.

The research was supported by the Russian Science Foundation, project No 16-14-10115.

SYNTHESIS OF HYALURONIC ACID RELATED OLIGISACCHARIDES

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Hyaluronic acid is a polysaccharide composed of repeating disaccharide units of β -D-glucuronic acid and N-acetyl- β -D-glucosamine. This biopolymer is a part of glycoproteins and proteoglycans that carry out important function in living organisms. It is notably that specific biological roles of hyaluronic acid are related to its molecular weight. The polymers from 100 to 10000 disaccharides units $\rightarrow 3$ - β -D-GlcNAc-(1 \rightarrow 4)- β -D-GlcA-(1 \rightarrow usually perform a structural role. They are components of the synovial fluid, the vitreous of the eye, the intercellular matrix. At the same time it has been reported, that small hyaluronic acid fragments carry out a regulatory role. They have an important role in essential biological process such as cell migration, proliferation, angiogenesis, inflammation and tumor development.

In this work stereoselective and regioselective synthesis of disaccharide 1 and trisaccharide 2 (Pic. 1), compounds related to hyaluronic acid, is described. The targeted compounds were synthesized from available monosaccharide precursors: D-glucose and D-glucosamine. Selective addition of protective groups was performed using modern methods of carbohydrates chemistry. For glycosylation the sulfoxide as donor was employed. Model compounds 1 and 2 are required for making clear of the mechanisms of regulatory action of hyaluronic acid.

