CONTENTS

EFFECT OF POLYSACCHARIDES FROM BROWN SEAWEEDS *LAMINARIA CICHORIOIDES* AND *FUCUS EVANESCENS* ON THE DEVELOPING SEA URCHIN EMBRIOS

Agarkova, V.V., Kiseleva, M.I., Ermakova, S.P., Zvyagintseva, T.N.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail:Vagarkova@mail.ru

Brown algae represent reach and easily regenerated source of polysaccharides: laminarans, fucoidans, and alginic acids. They have a wide spectrum of biological action and display antibacterial, anticoagulant, antithrombotic, anti-inflammatory, antitumor, contraceptive, and antiviral properties.

The purpose of this study was to examine an action of oligo- and polysaccharides from brown seaweeds *Laminaria cichorioides* and *Fucus evanescens,* aqueous-ethanol extract and inhibitors of 1,3-β-D-glucanases I_1 and I_2 from brown seaweed *L. cichorioides* on development and survival of the embryos of the sea urchin *Strongylocentrotus intermedius* in order to select more active compounds with stimulant and protective properties. To select compounds with contraceptive properties, we also examined the influence of these compounds on fertilization ability of sea urchin.

Sea urchins *S. intermedius* were collected from Peter the Great Bay, the Sea of Japan, Khasansky region, Prymorsky kraii, Russia. Brown seaweeds *L. cichorioides* and *F. evanescens* were collected in Troitsa Bay, the Sea of Japan and Iturup Island, Sea of Okhotsk, respectively. The abilities of four β-D-glucans, two fucoidans, and inhibitors I_1 and I_2 of protein nature to effect on embryos lifetime were tested.

Laminaran, glucan II, translam and fucoidan from *L. cichorioides* were only slightly active in this experiment. They have been showed to increase the duration of embryos life in 0.5 times. Heterogeneous in its monosaccharide composition fucoidan from *F. evanescens* revealed the greatest activity in this test and increased the duration of embryos life in 3 times.

After 0.5 day incubation, inhibitors I_1 and I_2 in low concentrations (0.015-0.025 mg/ml) decreased the lifetime on 50% in comparison with control. Aqueous-ethanol extract in concentration up to 0.5 mg/ml significantly reduced the viability of embryos, causing anomalies. The joint action of oligo- and polysaccharides and inhibitors I_1 and I_2 was studied and showed that all of the compounds studied reduced the inhibitory effect of I_2, increasing the lifetime of embryos.

We also investigated the influence of the compounds studied on the process of sea urchin fertilization. Oligo- and polysaccharides from *L. cichorioides* didn't inhibit this process. In contrast, polysaccharides from *F. evanescens* and inhibitors of 1,3-β-D-glucanases I_1 and I_2 had inhibitory effect on fertilization ability of sea urchin sperm. The contraceptive activities of the polysaccharides from *F. evanescens* and inhibitors of 1,3-β-D-glucanases I_1 and I_2 deserve further investigation to select potential natural spermicidal preparations.

EFFECTS OF THE METABOLITES FROM *KALANCHOE DAIGREMONTIANA* ON THE GROWTH OF SPROUTS OF AGRICULTURAL CROPS

[1]Anisimov, M.M., [2]Serebryakov, Yu.M., [1]Chaikina, E.L., [1]Gerasimenko, N.I., [2]Shtoll, I.R.
[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*
[2] *Seaside Research Veterinary Station, Vladivostok, Russia*
e-mail: anisimov@piboc.dvo.ru

Increase of efficiency of agricultural crops is very actually because cultivated areas are reducing significantly. One of the efficient methods to increase harvest is the application of natural growth regulators. Extracts of medicinal plants can be such growth regulators.

To reveal biological activity, in particular, regulating the growth of sprouts of corn and buckwheat, a group of substances of various chemical nature was isolated from *Kalanchoe daigremontiana*. To isolate these substances, the plant (500 g) was triply extracted with 96 % ethanol. From combined ethanol extract (EE), a lipid-pigment fraction (1.37 g) was then isolated by chloroform extraction. EE fraction was concentrated to minimum volume and treated with ethanol to obtain a precipitate of water-soluble polysaccharide I (0.82 g). After separating polysaccharide, the ethanol solution was used to obtain a complex of polyphenolic compounds (70 mg). The residual plant tissue was then extracted with 1 % Na_2CO_3 solution followed by the reprecipitation with ethanol to obtain polysaccharide II (5.7 g). Using column chromatography on silica gel, chlorophylls (22.3), carotenes (8.2), hydrocarbons (9.3), sterines (6), ethers of sterines (3,6), free fatty acids (FFA, 16.7), ethers of fatty acids (7.7), triglycerides (14), monogalactosyldiacylglycerol (MGDG, 3.4), sulfoquinovosyldiacylglycerol (SQDG, 3.7), and phospholipids (4.9) were isolated from the lipid-pigment fraction (% from weight of the fraction).

To evaluate activity stimulating the growth of sprouts, corn seeds were steeped in juice of *K. daigremontiana* for 24 hours, and then placed on a buttercloth moistened with water. The buckwheat seeds were couched in a roll of filter paper. The juice of *K. daigremontiana* was shown to stimulate the growth of a root of corn sprouts. In this experiment at juice concentration of 0.1 %, the root was 32.7 % longer than that in a control group.

Only 5 from 12 *K. daigremontiana* metabolites showed stimulatory effect on the growth of sprouts of buckwheat *Fagopyrum esculentum* Moench. (variety “Emerald”). EE is a full complex of substances from *K. daigremontiana*, including the water-soluble polysaccharides, lipids, pigments, mineral substances, amino acids, polyphenolic compounds, etc. At concentration of 0.01 μg/ml, this fraction stimulated the growth of the root and stalk on 8-10 %. The greatest amount of active substances falls on chlorophylls: 22.3 % from a total lipid fraction. This fraction stimulates the growth of the stalk by 8 % at concentration of 0.1 μg/ml. Polyphenolic compounds increase the growth of the root by 17 % at concentration of 1.0 μg/ml. FFA at concentration of 0.1 μg/ml stimulate the growth both of the root and of the stalk by 8-13 %, respectively. Triglycerides increase the growth of the stalk only by 10 % at concentration of 100 μg/ml.

Thus, the data obtained showed that the juice from *K. daigremontiana* stimulated the growth of sprouts of corn. EE as a whole, its polyphenolic fraction, and separate components of the polyphenolic fraction are of interest as the substances with stimulatory activity towards the growth of sprouts of the buckwheat *F. esculentum* Moench. (variety “Emerald”). EE from *K. daigremontiana* can also be applied in agriculture to stimulate the growth activity of corn and buckwheat.

ENVIRONMENTAL GROWTH CONDITIONS INFLUENCE ON THE BIOLOGICAL PROPERTIES OF *YERSINIA PSEUDOTUBERCULOSIS*

[1]Bakholdina, S.I., [2]Shubin, F.N., [1]Solov'eva, T.F.
[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*
[2]*PI SRI of Epidemiology and Microbiology RAMS, Sel'skaya, 1,Vladivostok*
e-mail: bakholdina@piboc.dvo.ru

Yersinia pseudotuberculosis enteroinvasive human pathogen can survive outside of a host organism for a long time under rather low temperature. Earlier it was shown that virulence of pseudotuberculosis bacteria can increase under growth in these conditions. However, the environmental conditions giving rise to this phenomenon are unknown. They can be complex, as bacteria are influenced by variety of physical and chemical factors in abiotic environment. The study of growth factors, which together with low temperature contribute to formation of the potentially virulence phenotype of bacteria, have theoretical and practical interest. In current work we studied the influence of oxygen availability and presence of glucose and galactose in growth medium at 8 °C on biological properties of pseudotuberculosis bacteria and also on their resistance to antibiotics, phenol and heat stress under an abrupt rise in the growth temperature from 8 to 37 °C.

The adhesive and invasive activities of *Y. pseudotuberculosis* were shown to increase during their growth at 8 °C under an oxygen-poor environment (without stirring the medium). Bacteria, grown under these conditions, survive an abrupt rise in the growth temperature from 8 to 37° C in contrast with cells, grown under intense aeration conditions. In nature such a transition from low to high environmental temperatures takes place when saprophytic bacteria go to parasitic life. Irrespective of aeration conditions the galactose added to growth medium enhances the cell resistance to the toxic effect of phenol (two-fold increase of 50% bacterial cell-permeabilizing phenol concentration), while the glucose inhibits invasive activity of bacteria and decreases their resistance to ciprofloxacin.

Thus, the galactose presence and oxygen deficiency in the cultural medium under low growth temperature will aid in increasing *Y. pseudotuberculosis* pathogenic potential. The results present an interest for solving practical aims, connected with pseudotuberculosis infection prophylactics and also with the processing and keeping foodstuff.

O-GLYCOSIDE HYDROLASES OF MARINE BACTERIA AND PROSPECTES FOR THEIR APPLICATION IN BIOTECHNOLOGY

Bakunina, I.Yu.

Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, 159, prospect 100 let Vladivostoku, Vladivostok, 690022, Russia, tel. (4232)310305,
e-mail: bakun@mail.ru

O-glycoside hydrolases are enzymes, catalyzing hydrolytic disruption of O-glycoside bounds in carbohydrates and carbohydrate containing biopolymers. These enzymes are demanded by modern biotechnology, producing different enzymes for food industry, pharmacology, light industry, medicine and other field needs. O-glycoside hydrolases are key enzymes of carbohydrate metabolism in high organisms, plants and microorganisms. In this connection knowledge about their properties, structure and mechanism of action is useful for understanding of many cells' processes. Furthermore, glycoside hydrolases are helpful and effective instruments for structure analysis of corresponding natural polysaccharides and carbohydrate containing biopolymers.

The present report summarizes knowledge on the different marine bacteria O-glycosyl hydrolases, involved in cell wall antigens and brown seaweed polysaccharide degradation. The data on distribution among symbiotic and free-living marine bacteria are shown for α-N-acetylgalactosaminidases and α-galactosidases, as well as fucoidanases, enzymes, digesting sulfated fucose-containing polysaccharides from brown macroalgae.

α-Galactosidase, α-N-acetylgalactosaminidase, converting group specificity of A and B erythrocytes of human blood were isolated from marine bacterium *Pseudoalteromonas* sp. KMM 701 and *Arenibacter latericius* KMM 426, comparatively. The enzymes were purified and characterized in detail (MW, pH optimum, T stability, inhibition of α-galactosidase with natural and synthetic compounds). The elements of structure, hydrolytic properties and substrate specificity of these enzymes were studied. Action of the enzymes on cells of human A and B erythrocytes and buccal epithelium was investigated. Properties were studied for free and immobilized into hybrid polysaccharide-silica nanocomposite materials α-galactosidase of marine bacterium *Pseudoalteromonas* sp. KMM 701.

Fucoidanases were isolated from marine bacteria *Pseudoalteromonas citrea* KMM 3296, 3298 and 3297. These enzymes degraded the α-(1→3,1→4)-fucan of brown algae *Fucus evanescens* and α-(1→3)-fucan of *Laminaria cichorioides* at pH 6.5-7.0 and remained active at 40-50°C. The endo-type hydrolysis of fucoidans resulted in the formation of sulfated di-, three-, tetra- and pentafucooligosaccharides. The enzymes were shown to catalyze strongly the cleavage of accessible α-(1→3)-L-fucoside bonds in the both fucans. Fucoidanase from marine bacteria *Mesonia algae* KMM 3909^T on the contrary catalyzed the degradation of α-(1→3,1→4)-fucan of brown algae *F. evanescens* only. As shown by size-exclusion chromatography, ^1H and ^{13}C NMR spectra of the main product of enzyme depolymerization, the fucoidanase from *M. algae* KMM 3909^T proceeds according to an endolytic mode and cleaves the α-(1→4)-glycosidic linkages.

Possible biotechnological application of the enzymes is discussed.

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EFFICACY OF THE TREATMENT CHUM SALMON EGG WITH GLUCANS IN PREVENTION OF SAPROLEGNIOSIS

Balabanova, L, Kiseleva, M, Elyakova, L, Rasskazov, V, Zvyagintseva, T.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: balaban@piboc.dvo.ru

Chum salmon, *Onchorhynchus keta* (Walbaum), were hampered by approximately 10 to 25% mortality caused by *Saprolegnia* infection during every hatching season on the Far-Eastern fish farm "Razanovsky". It is known that the immune system of fish is not fully competent until 2-3 months after hatching, and the larvae are wholly reliant on non-specific parameters for their defence against infection. There is set of substances derived from bacteria and fungi, which can activate these parameters. Marine alga polysaccharides were found to be also effective immunostimulants similarly to yeast or bacterial glucans. The effect of several 1,3;1,6-β-D-glucooligo- and polysaccharides from brown algae, *Laminaria cichorioides,* with different structures (from 1 to 10 kDa of molecular mass; from 10 % to 25 % of β-1,6-linked glucose residues content) on the developing chum salmon was evaluated using the different methods of egg treatments. The chum salmon cultures developed from the eggs treated with 1,3;1,6-β-D-glucans were monitored over 6 years for developmental peculiarities or mortality, and their ability to be infected by *Saprolegnia* spp. The parameters such as mortality rates of embryos and juveniles (%), amount of the eggs spontaneously affected by *Saprolegnia* spp. (%), alteration in the body weight of juveniles during incubation period, and change of anti-B lectin titre in the embryo extracts were observed at the key points of development in comparison with the controls untreated with 1,3;1,6-β-D-glucans. The immunity of fish was evaluated with registration of the *Saprolegnia* infection events and determination of anti-B lectin titre in the embryo extracts at the ocellus stage on 10 and 45 days of development. All 1,3; 1,6-β-D-glucans have been proved to be very effective on developing juveniles and distinct each from other by stimulating doses and efficacy independent on the method and time of treatment. Exposure of the eggs to the solutions of 1,3;1,6-β-D-glucans with the molecular mass of more than 2 kDa increased embryos' and juveniles' survival and resistance to *Saprolegnia* infection up to 2.5-fold that led up to the weight increment of juveniles by 40-55% compared to the control fish. 1,3;1,6-β-D-glucans with a molecular mass of between 6-8 kDa at concentration of 0.5 mg/ml showed the best stimulative effect on the developing chum salmon. Thus, the egg treatments with laminaran decreased the events of juveniles' mortality 2.5-fold. However, at the earlier period of the development, translam and glucan IV showed the best results that led up to reduction in the juveniles' mortality by half as compared to the controls. As was shown earlier, fucoidans, in distinction from 1,3;1,6-β-D-glucans, should be carefully used for egg treatments because of their inhibitory action on embryo developments in high concentrations. The lowest rate of *Saprolegnia* infection and embryos' mortality were observed in the cultures developed from the eggs, which were immediately treated with 1,3;1,6-β-D-glucans after fertilization. Preliminary exposure of the unfertilized eggs to the 1,3;1,6-β-D-glucan solutions had a negative effect on further fertilization. However, in the case of succeeded fertilization, the eggs and embryos were rarely affected by saprolegniosis in comparison with the controls. The biological activity of 1,3;1,6-β-D-glucans was confirmed by increment of anti-B lectin titre in all embryo extracts. The data of this study allow us to recommend fish egg treatments with the use of more accessible laminaran for prevention of saprolegniosis in fish-culture.

MOLECULAR POPULATION GENETICS OF *BACTEROIDETES* SYMBIONTS IN THE SEA URCHIN *STRONGYLOCENTROTUS INTERMEDIUS*

[1, 2]Balakirev, E. S.,[2]Ayala, F. J.
[1]*Institute of Marine Biology, Vladivostok 690041, Russia*
E-mail address: esbalakirev@mail.ru
[2]*Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697-2525, USA*
e-mail: fjayala@uci.edu

Strongylocentrotus intermedius (A. Agassiz 1863) is an economically important sea urchin inhabiting the northwest Pacific region. The northern Primorye (Sea of Japan) populations of *S. intermedius* consist of two sympatric morphological forms, "usual" (U) and "grey" (G). The two forms are significantly different in morphology and preferred bathymetric distribution. Bacteria of the genus *Tenacibaculum* (phylum *Bacteroidetes*) have been reported to be agents of the sea urchin *S. intermedius* gonad spotting disease. We have investigated the bacterial infective agents present in *S. intermedius* by means of 16S rRNA sequences with primers located in a conservative region of the bacterial gene to amplify the gene from representatives of the genus *Tenacibaculum* and related bacteria. For comparative purposes we have also analyzed the bacterial composition in another sea urchin species, *S. nudus*. We have amplified, cloned, and sequenced the fragments (1360 bp) of the bacterial 16S rRNA gene for 49 individuals of *S. intermedius* and nine individuals of *S. nudus* (totally 325 clones) from three distant localities of the northern Primorye region. A BLAST search of each clone found close matches with multiple bacteria belonging to the phylum *Bacteroidetes*. Most of the inferred bacteria are members of two classes: *Flavobacteria* and *Sphingobacteria*; two sequences were associated with a third class, *Bacteroidetes*. The *Flavobacteria* clones match representatives from seven genera of the family *Flavobacteriaceae*: *Elizabethkingia*, *Capnocytophaga*, *Lutibacter*, *Bizionia*, *Flavobacterium*, *Formosa*, and *Winogradskyella*. The *Sphingobacteria* clones match representatives from the single genus *Cytophaga* (family *Flexibacteriaceae*). The level of divergence between the *Cytophaga* phylotypes is actually very similar to the divergence between different genera within the family *Flavobacteriaceae*. This observation may reflect relatively poor taxonomical knowledge of *Sphingobacteria* in comparison with *Flavobacteria*. We have detected no *Tenacibaculum* infection in *S. intermedius* from the northern Primorye. However we have observed two previously not described phylotypes both for *Flavobacteria* and *Sphingobacteria* exhibiting the epidemic pattern of phylogeny: 1) the phylotypes include a major part of the bacterial clones; 2) the level of genetic variability is low; 3) mutations are mostly represented by singletons; 4) genetic distance between the clones is low; 5) the pattern of variability significantly deviates from neutral expectation; 6) recombination and gene conversion is low. The observed population genetics pattern (which is very similar in three distantly located settlements of *S. intermedius*) indicates epidemic spreading of the two *S. intermedius* phylotypes and is consistent with the expected effects of genetic drift under the repeated bottlenecking caused by vertical bacterial transmission. Importantly, the prevalent *Bacteroidetes* phylotypes are differentially associated with the U and G morphological forms of *S. intermedius*: the *Flavobacteria* phylotype is mostly associated with the U form and the *Sphingobacteria* phylotype is mostly associated with the G form. However the bacterial sequences from *S. nudus* are intermingled among the sequences from *S. intermedius*, independently of the depth at which they were collected, indicating that the depth of the sea urchin settlements by itself does not play a determinant role in structuring the bacterial communities. Thus, *Bacteroidetes* symbionts are widespread in *S. intermedius* and, moreover, they have different distribution in the U and G morphological forms. Consequently, symbiont-induced life history changes may have promoted environmental specialization and might potentially promote speciation in *S. intermedius*. If so, and given the evidence that symbiont-associated changes in dispersal and mating are likely to play a key role in the initiation of genetic differentiation of populations with different infections, it might be the case that the U and G forms could be considered incipient species, even though their divergence may have occurred recently. We propose that the *Bacteroidetes* endosymbiotic bacteria could be an important causative factor leading to morphological and potentially genetical divergence of sea urchin *S. intermedius*.

THE RED SEAWEED *TICHOCARPUS CRINITUS* (TICHOCARPACEAE) IS SOURCE OF A NEW TYPE OF CARRAGEENAN

Barabanova, A.O., Yermak, I.M., Glazunov, V.P., Isakov, V.V.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: anuta@piboc.dvo.ru

Sulfated polysaccharides occurring in the red algae *Tichocarpus crinitus* cell wall were fractionated and purified. NMR and FT-IR spectroscopy analyses revealed that the non-gelling fraction contained a sulfated galactans having a new carrageenan-like structure (Figure). It is built with alternatively linked 1,3-linked β-D-galactopyranosyl-2,4-disulphates and 1,4-linked 3,6-anhydro-α-D-galactopyranosyl residues. Minor amounts of its biosynthetic precursor were detected in water extracted specimen. Brief analysis of rheological and biological properties of the non-gelling fraction was carried out. The carrageenan-like polysaccharide from *T. crinitus* displayed the properties of "random coil" polymer at high temperature and possesses high anticoagulant activity at low concentration.

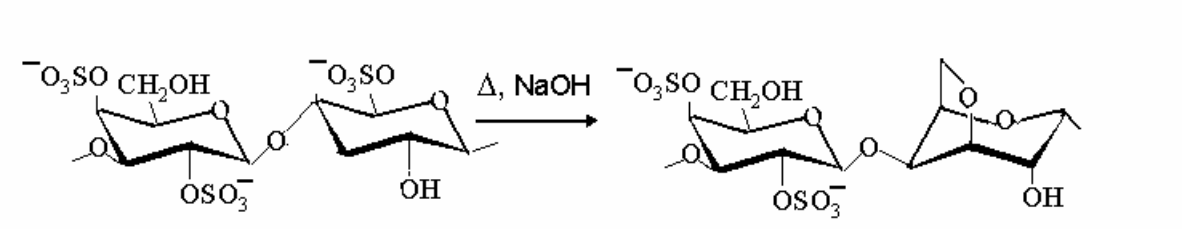


Figure.The proposed structure for the non-gelling polysaccharide from *T. crinitus* and its biosynthetic precursor

INTRAMOLECULAR DYNAMICS AS A FACTOR DETERMINING MAGNETIC PROPERTIES OF MOLECULES

Berdyshev, D.V.[1], Izrailskii, Yu.G.[2], Novikov, V.L. [1], Glazunov, V.P. [1]

[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*

E-mail: *glazunov@piboc.dvo.ru*

[2] *The Institute of Automation and Control Processes, 690041, Vladivostok, ul. Radio, 5,*

NMR spectroscopy is a powerful tool for probing the structure and dynamic properties of compounds. There are a rich variety of experimental data showing the intimate dependence between parameters of NMR spectra of various molecules and their intra- and intermolecular dynamics Here we present the results of the study of influence of intramolecular dynamics in compounds **1** and **2**, the simplest analogs of unique natural β,β′-triketones, on the chemical shifts of various nucleus. Three types of large amplitude motions (LAM) can proceed in these molecules: intramolecular hydrogen atom transfer causing keto-enol and enol-enol tautomerism; internal rotation of C9-OH group in tautomeric form **b** coupled to the rotation of ethylidene group about C2=C9 double bond or rotation of C1-OH group in tautomeric form **a** coupled to the rotation of COMe group about C2–C9 ordinary bond, leading to convertion from form "up" to form "down"; internal rotation of two Me groups leading to modulations in energetic characteristics of the potential energy surface (PES) for intramolecular O–H…O hydrogen bond.[1] Experimental ^{1}H and ^{13}C NMR spectra for **1** exhibit two groups of corresponding signals, with relative intensity $k \approx 3 : 2$. The reason of this doubling may be or tautomeric equilibrium between forms **a** and **b** for the main isomeric form ("up" or "down"), or the equilibrium between isomeric forms themselves, so that $k = (\mathbf{a}^{\mathbf{down}} + \mathbf{b}^{\mathbf{down}})/(\mathbf{a}^{\mathbf{up}} + \mathbf{b}^{\mathbf{up}})$. It was shown in our previous work, that for **1** and **2** global minimums on PES correspond to "di-keto" tautomeric form **b**. The energies of "tri-keto" forms are about 7-8 kcal•mol^{-1} higher then energies of forms **b** and 2-3 kcal•mol^{-1} higher, then of forms **a**.[1] Since that in present investigation we do not account for all tri-keto forms and di-keto forms of type **a**, as their amount is less then 0.01%. So only **b** forms notably contribute to NMR spectra.

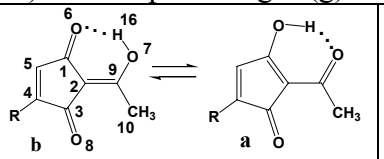
The conformational analysis of **1**, concerning the internal rotations of methyl groups (Me1 and Me2), was carried out using B3LYP/cc-pVTZ level of theory. After the full geometry optimization of rotamers was done, the followed normal-mode analysis showed that only rotamers **db-ct**, **ub-ct** and **da-ct**, **ua-ct** correspond to minimums on PES. But as all other rotameric forms can be obtained via internal rotations, which proceed in potentials with very low barriers (Table 1), their energies were taken into account when we calculated percentages of forms **db-ct**, **ub-ct**. This leads to a more correct estimation of percentages for "up" and "down" isomers, since this in approximate manner accounts for the populations of vibrational levels for the Me1 and Me2 internal rotations. We then calculated one-dimensional (1D) potential functions for the internal rotations of Me1 and Me2 groups as PES scans along the minimum energy paths (MEP) in mass-weighted Cartesian coordinates, and solved variationally one-dimensional Schrödinger equations for the movements of a particle with m=1 a.m.u. in these potentials. The dependencies of NMR shielding constants from the position on the MEPs were calculated with B3LYP/6-31G(d) method and averaged over vibrational distribution functions, calculated for 1D tasks (see Table 2).

One can see that rotation of Me1 group (LAM1) influences more profoundly on $\sigma(^{13}C9)$ and $\sigma(^{13}C10)$, then the rotation of Me2 group (LAM2). On the other hand, LAM2 influences on $\sigma(^{13}C2)$, $\sigma(^{13}C3)$ and $\sigma(^{13}C10)$ is the main. This effect however can not be the reason of the changes in the chemical shifts $\Delta\delta(C4)=\delta^{\mathbf{d}}(C4) - \delta^{\mathbf{u}}(C4)=17.3$ ppm and $\Delta\delta(C5)=\delta^{\mathbf{d}}(C5) - \delta^{\mathbf{u}}(C5)=- 17.2$ ppm,

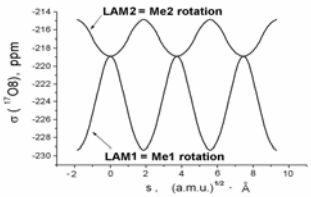
which is two order of magnitude bigger, then the value of contribution from internal rotations to $\sigma(^{13}C4,5)$. So, experimental $\Delta\delta_{4\to5}$ value is the result of "down"↔"up" conversion. The contribution of Me's rotations on $\sigma(^{1}H16)$ are less then 0.05 ppm. It is seen from table 2, that the most "conformationally-sensitive nucleus" is the O8.

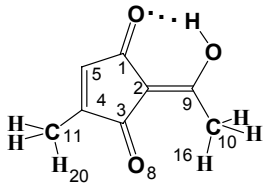
Table 1. The values of energy (E_0, kcal•mol^{-1}) and the percentages (g) of isomers **1b**.

Isomeric form*	6-31G(d)		cc-pVTZ	
	$-E_0$	g ,%	$-E_0$	g ,%
db-ct	0.00	41.6	0.00	44.3
ub-ct	0.39	21.5	0.42	21.5
db-cc	0.53	16.9	0.73	12.8
ub-ct	1.03	7.3	1.21	5.7
db-tt	1.14	6.1	1.01	8.1
ub-tt	1.54	3.1	1.43	4.0
db-tc	1.66	2.5	2.22	1.0
ub-tc	2.18	1.0	1.71	2.5

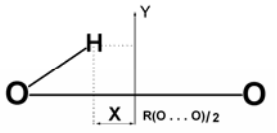


1: R=Me; **2**: R= H
(for **1**: C9-Me≡Me1 and C4-Me ≡Me2)





db-cc



coordinate system for 2D task

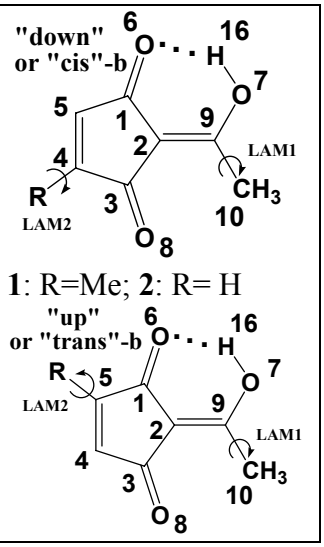
* The 4-symbol notation of the rotameric forms* was used: **γη–αβ**, where **γ=d** or **u**, **η=b** or **a**, $\alpha \equiv \angle$H16-C10-C9-C2, $\beta^{d} \equiv \angle$H20-C11-C4-C3 or $\beta^{u} \equiv \angle$H20-C11-C5-C1 take values 0.0° ≡ **"c"** or 180.0° ≡ **"t"**

To evaluate the contribution, which gives the vibrational motion of the bridged proton H16 to its isotropic NMR shielding constant in **2**, the two-dimensional (2D) PES scan was done to construct the 2D diabatic potential V(x,y), after which the 2D Schrödinger equation was solved numerically using Lanczos method on a fine grid. The obtained 2D wavefunctions were used for the averaging of 2D function $\sigma(^{1}H16)=f(x,y)$. The obtained 2D ground state contribution to $\langle\sigma(^{1}H16)\rangle$ is: $\Delta\langle\sigma(^{1}H16)\rangle^{2D} = \langle\sigma(^{1}H16)\rangle^{2D} - \sigma$(eq; **b**) = 19.99 - 20.64 = -0.65 ppm.

This work was done with a partial financial support of the FEBRAS grant №06ПУО01001-part 2.

Table 2. LAM contribution to relative chemical shifts (in ppm)♥)

Nucleus	δ_{calc}		LAM1 $\Delta\delta_{calc}$♦)	LAM2 $\Delta\delta_{calc}$♦)	$\langle\delta_{calc}\rangle$♦)	δ_{exp}	
	ub-ct	**db-ct**				«down»	"up"
C1	177.30	175.97	-0.115	0.108	175.00	181.9	182.4
C2	85.80	85.70	-0.098	-0.492	84.146	86.2	86.4
C3	166.03	167.98	-0.004	-0.363	166.65	174.1	173.6
C4	118.57	137.15	-0.159	-0.414	135.61	139.7	122.4
C5	131.28	113.40	0.0296	0.0056	112.47	118.1	135.3
C9	154.00	154.26	-0.219	0.092	153.16	159.0	158.8
C10	0.0	0.00	0.967	-0.003	0.00	0.0	0.0
	σ_{calc}	σ_{calc}	$\Delta\sigma_{calc}$	$\Delta\sigma_{calc}$	$\langle\sigma_{calc}\rangle$		
O6	-135.60	-142.17	0.59	0.13	-141.44		
O7	168.97	165.91	-0.59	0.01	165.33		
O8	-234.1	-218.89	-2.77	1.49	-220.17		
H16	20.65	20.56	0.02	-0.02	20.56		



1: R=Me; **2**: R= H

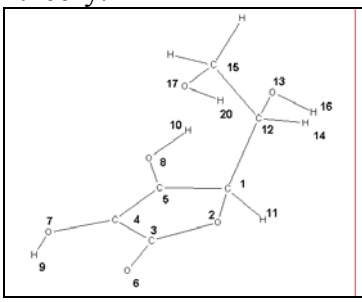
♥) $\delta_{calc} = \sigma(^{13}C_i) - \sigma(^{13}C10)$; $\Delta\delta_{calc}$ – LAM contribution to δ_{calc} ; δ_{exp} (^{13}C10)=18.2 ppm.; $\sigma_{calc}(^{13}C10,down)$=173.47 ppm; $\sigma_{calc}(^{13}C10,up)$=173.30 ppm. ♦) only for the **db-ct** isomer.

1. Berdyshev D.V., Glazunov V.P., Novikov V.L. - VI All-Russian scientific seminar and Youth scientific school "Chemistry and Medicine", Ufa, 26-29 November 2007, pp. 133-135.

THEORETICAL TREATMENT OF THE CONFORMATIONAL MOBILITY AND VCD SPECTRA OF L-ASCORBIC ACID

Berdyshev, D.V.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
Fax: +7 (4232) 314050. E-mail: dberdyshev@piboc.dvo.ru

The conformational analysis of L-ascorbic acid (**L-AsA**) was done using B3LYP/cc-pVTZ level of theory.



The notation was used: **xyzw-r**, where **"x","y","z"** ,**"w"** and **"r"** are the types of angle values*), correspondingly for:
$\theta_{13}\equiv\angle$13-12-1-2; $\theta_{16}\equiv\angle$16-13-12-1; $\theta_{17}\equiv\angle$17-15-12-1;
$\theta_{20}\equiv\angle$20-17-15-12; $\beta\equiv\angle$10-8-5-4;
*)**b**– [0.0°≤θ≤120.0°]; **a**– [-120.0°≤θ≤0.0°]; **c**– [120.0°≤θ≤240.0°];
r- [90.0°≤β≤270.0°]; **u-** [-90.0°≤β≤90.0°];

First, 162 starting conformations were considered for full geometry optimization with B3LYP/6-31G(d) method, followed by thermochemistry analysis. As a result, 114 different rotameric forms of **L-AsA** were obtained, among which there are 9 main conformers, which total amount is more then 98%. The Gibbs free energies for them differ less then about 2 kcal mol^{-1}. These conformers as well as five nearest to them on the energy scale were then considered for full geometry optimization and for thermochemistry analysis with B3LYP/cc-pVTZ method.

Table 1. Electronic energies (E_0/a.u.), zero-point vibrational energies (*ZPE* /a.u.), relative electronic energies (ΔE_0/kcal mol^{-1}), relative Gibbs energies (ΔG / kcal mol^{-1}) and percentages ($g^{\%}$/%) of the main rotameric forms, calculated with B3LYP/cc-pVTZ method.

Rotamer	$-E_0$	*ZPE*	ΔE_0	ΔG	$g^{\%}(E_0)$	$g^{\%}(G)$
babb-r	685.043412	0.114483	0.05	0.04	23.52	18.31
cbab-r	685.040239	0.11473	2.04	2.18	0.82	0.49
baaa-u	685.042189	0.113529	0.81	0.20	6.44	13.78
abab-u	685.042936	0.113980	0.34	0.02	14.22	18.85
babc-r	685.042969	0.113984	0.32	0.00	14.72	19.42
baac-r	685.041425	0.114758	1.29	1.45	2.87	1.67
cccc-r	685.04226	0.113932	0.77	0.41	6.94	9.68
baaa-r	685.043484	0.114935	0.00	0.27	25.39	12.24
ccca-r	685.041964	0.114162	0.95	0.74	5.08	5.55

One can see from table 1, that three most stable conformations have practically the same Gibbs free energy, two of them correspond to "**r**"-type, and the third – to "**u**" type. The role of entropy in the order of rotamer's relative stability is high. Our results perform a new row of relative stability for **L-AsA** conformations, because earlier the **baaa-r** conformation was considered in literature to be the most stable in gas-phase.

The complexes of **L-AsA** with five water molecules were then modeled with B3LYP/cc-pVTZ method. It was found, that the most stable conformations are of "**u**" type, and that the most energetically preferable conformations realize, when all hydroxyl groups are involved in the intermolecular hydrogen bonds with water molecules in such a manner, that leads to creation of a chain of O-H…O bonds, in which the links lie approximately in one and the same plain. Water molecules due to their high rotational mobility play a role of modulators, which dynamics governs the conformational preferences and, hence, the reactivity of the solvated **L-AsA**.

CRYORESISTANCE STUDY OF MARINE HYDROBIONTS AND CRYOBANK FOUNDATION

[1]Boroda, A.V., Odintsova, N.A., [2] Kostetsky, E.Y.

[1]*A.V. Zhirmunsky Institute of Marine Biology, Far Eastern Branch of Russian Academy of Sciences, 17 Palchevsky Str., Vladivostok, Russia;*

[2] *Far-Eastern National University, Vladivostok, Russia*

Tel. (4232) 31-09-43; Fax (4232) 31-09-00

e-mail: avboroda@yandex.ru

The project is aimed to developing of cryopreservation procedures for marine hydrobionts at cryopreservation in liquid nitrogen and designing of new cryoprotectors. Damages of cell membranes and free radical production are considered to be principal factors in cold shock injury of cells. Peculiar fatty acid composition of cell membranes of marine animals and plants, in which prevail unsaturated fatty acids, has a significant influence on cryoresistance of their cells and requires new cryoprotectors in principle. The objective of the current study was to increase the extent of integrity and functional activity of marine invertebrate cells after freezing-thawing by maintenance of their membrane stability. We are going to study thermotropic behavior of marine hydrobiont lipid extracts, analyze lipid composition of cell membranes before and after freezing-thawing, test the cryoprotective properties of exogenous lipids in complex with membrane stabilizers and antioxidants. We consider that it is possible to significantly reduce the loss in biological activity of marine invertebrate cells after the thawing due to synergy activity of these components. By developing of this direction, a basis is provided for future studies geared at better understanding cryoresistance mechanisms of marine invertebrate cells, founding of modern Cryobank of reproductive products, embryos, larvae and cells of these animals and for marine biothechnology.

A NEW GROUP OF MANNAN-BINDING LECTINS OF THE ECHINODERMS

Bulgakov, A. A.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: aabulgakov@yahoo.com

C-type mannan-binding lectins (MBLs) are an important component of innate immunity, responsible for the "first line" of host defense. As was shown for vertebrate animals, MBLs mediate recognition and elimination of the pathogens expressing mannose-rich glycoconjugates on their surface.

The Echinoderms are evolved during more then 400 millions years and developed the effective innate immune system. During evolution, a lectin-mediated defense mechanism preceded the appearance of an integral cellular and humoral immune response of vertebrates. Recent advances in the comparative immunology strongly support the conception that the immune system of all modern animals has the roots in very ancient organisms. Based on this conclusion, we tried to find precursor of the MBLs family in echinoderms, the group of marine invertebrates (Deuterostomata) that belongs to the same evolutionary line as all vertebrates.

By the moment of the beginning of our researches, only Ca^{2+}-dependent and independent Gal-specific, GalNAc-specific and specific to uronic acids lectins have been isolated from the Echinoderms.

Screening of the local echinoderms for mannan-specific lectins revealed their presence in the holothurians *Cucumaria japonica, Apostichopus japonicus,* sea urchins *Strongylocentrotus nudus, Strongylocentrotus intermedius* and starfishes *Echinocardium cordatum*, *Distolasteria Nippon, Asterias amurensis, Asterina pectinifera*. Result of this search was successful, because as ligands for investigation of lectins carbohydrate specificity we have used polysaccharides isolated from marine bacteria. As a result, four new C-type lectins strong reacting with high branched α-D-mannans representing glycoconjugates with α-1,2 and α-1,6 linked D-mannopyranose and fucose residues were isolated. Physicochemical, immunochemical and structural studies and response to injection of pathogenic microorganisms were carried out for the MBL-CJ, MBL-AJ and MBL-SN.

Isolated MBLs were shown to be structurally and functionally similar to vertebrate MBLs. The major evidence for this conclusion is their carbohydrate specificity, the presence in their structures of antigen determinants common with human MBL and conserved region forming the mannose-binding site, and participation in defense reactions. Taken together, these properties suggest that the mannan-binding lectins from the investigated holothurians *A. japonicus, C. japonica* and sea urchins *S. nudus, S. intermedius* are phylogenetically related to an evolutionary precursor of vertebrate MBLs and that MBLs are the family of highly evolutionary conservative mannan-binding proteins.

The mannan-binding lectins of the echinoderms are interesting for development of diagnostic methods of oncological diseases because they do not interact with normal components of human plasma of healthy donors. This conclusion has been supported by development of the mannan-binding lectin based immunoassay for the cervical cancer diagnosis.

DISTRIBUTION AND PROPERTIES OF SOME O-GLYCOSYLHYDROLASES FROM MARINE FILAMENTOUS FUNGI

Burtseva, Yu.V., Sova, V.V., Pivkin, M.V., Zvyagintseva, T.N.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: burtseva@piboc.dvo.ru

The main polysaccharides of brown algae (laminarans, fucoidans and alginic acids) are biologically activity substances. Seaweeds are well known to be colonized by marine fungi. O-Glycosylhydrolases of marine fungi are capable to degradate carbohydrates and play an important role in the breakdown of seaweeds. Enzymatic degradation of carbohydrates is the basis of many biotechnological processes. Individual enzymes have been successfully employed for establishment of the structure and biological role of polysaccharides.

Marine fungi are less investigated than other ecological groups. Obligatory marine fungi are still main objects. However, recently interest in studying marine facultative fungi has been growing since many new metabolites have been isolated from them which have not been found in terrestrial fungi. Among the metabolites of marine fungi, enzymes degrading polysaccharides are of a special interest. It has been shown, that marine fungi possess effective and various enzyme systems. Marine fungi are widely used as sources of enzymes. Studies of marine fungi enzymatic systems present great theoretical and practical interest. Systematic analysis of the composition and level of O-glycosylhydrolase activity of marine filamentous fungi has not previously been conducted. The small numbers of papers are devoted to O-glycosylhydrolases of marine fungi.

We conduct systematic search among marine filamentous fungi a new producers of high active O-glycosylhydrolases which possess appointed specificity and stability at various negative factors. The O-glycosylhydrolases composition of 195 marine filamentous fungi strains was studied. These fungi were collected near Kuril Islands (34 strains) and in Pociet Bay of the Sea of Japan (71 strains), as well as in the Sea of Okhotsk (76 strains) and in the territorial waters of the Socialist Republic of Vietnam (19 strains) from different marine habitats (bottom deposits and associated with brown algae and marine invertebrates). It has been shown, that strains mainly produce four enzymes: 1,3-β-D-glucanase, amylase, N-Acetyl-β-D-glucosaminidase and β-D-glucosidase. The enzymes hydrolyzing agar, galactan, and α-D-fucopiranoside were not found. The differences in the composition and the level of O-glycosylhydrolase activity in cultural liquid and mycelium of marine fungi were exposed. Thus our studied allowed us to reveal a series of strains promising as producers both of individual enzymes and of a set of enzymes splitting carbohydrate-containing compounds.

The dependence of enzyme synthesis from carbon source was studied to select optimal conditions for the growth and producing of high active O-glycosylhydrolases. The biosynthesis of enzymes was activated by addition of laminaran in the nutrition medium. Methods of gel-filtration, ultrafiltration and ionexchange chromatography were used for the purification of O-glycosylhydrolases from marine fungi.

It is shown that 1,3-β-D-glucanases *Chaetomium indicum* and *Trichoderma aureviride* are specific to *β*-1,3-bonds in glucans, release predominantly glucose from laminaran and do not catalyze reaction of transglycosylation. Accoding to these data enzymes are exo-glucanases. Inhibitor analysis demonstrated the significant role of tryptophan and tyrosine residues in the catalytic activity of enzymes. Molecules of *T. aureviride* laminarinase contained the functionally important thiol group. Some enzymatic properties of β-D-glucosidase, 1,3-β-D-glucanase, amylase, N-Acetyl-β-D-glucosaminidase from *Penicillium fuscum* and *P. canescens* were studied. 1,3-β-D-Glucanase of *P. fuscum* was classified as endo-enzyme.

This work was supported by grants of FEB RAS № 06-III-B-05-127 and RFFR № 06-04-48540-a and № 08-04-00289-a, “Molecular and cell Biology”.

INVESTIGATION OF LIPID FRACTION OF THE SEA WORM *URECHIS UNICINCTUS*

Busarova, N.G., Revtsova, I.U., Isay, S.V.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: isai@piboc.dvo.ru

Lipids and their natural complexes are not only the basic components of biological membranes, but also regulators of activity of some enzymes and signal compounds. Sea organisms are rich lipid sources. Among the big variety of researched sea organisms, worms are investigated to a lesser degree. Sea worms (SW) are the important part of a food circuit. For example, SW *Urechis unicinctus* enters into a diet of native peoples of the Far East, is fodder object of some food fishes, and also, alongside with urchins, carry out a role of model in genetic researches. So, discovery of disconnect of the RNA-pigment, playing the important role not only in immune protection, but also in regulation of protein synthesis performed on SW *Caenorhabditis elegans*, was awarded the Nobel Prize (1).

SW *U. unicinctus*, caught on Institute marine experimental station in Peter the Great bay of Japan sea was object of our research. Lipid fractions of the skin-muscular bag (SMB), blood, internals, and gonads were investigated. Total lipids in the indicated tissues have made 5.9, 14.8, 34.9 and 37.4 % (on dry weight of a tissue), respectively. Two-dimensional TLC has shown that all samples investigated contain a set of the following phospholipids (PL): phosphatidylethanolamine (PE), lyso-phosphatidylethanolamine (LPE), phosphatidylcholine (PC), diphosphatidylglycerol (DPG), phosphatidylinositol (PI), phosphatidylserine (PS). PC and PE were the major PL that is in agreement with the literature data (2). Besides, in three samples (except for blood) sphingomyelin (SM) with the contents of 0.4-3.3% was found out. Gonads and internals are the most rich in PL content, 53.1 and 51.4 %, respectively. PL content in blood is 32.3 %, in SMB - 19.8 %. The contents of neutral lipids (NL) are practically in equal proportion to PL contents in gonads and internals and their amounts are essentially higher in blood and SMB. In all tissues, the major components of NL were triglycerides and cholesterol.

We investigated also composition and proportions of fatty acids (FA) in tissues of *U. unicinctus*, as modern medical hypotheses connect many illnesses of the person with deficiency of polyunsaturated FA (PUFA) in an organism. It is necessary to note, that contents of PUFA in *U. unicinctus* essentially differ from a species to a species that can be connected with time (season) of catching (3, 4). In the tissues investigated, FA with odd number of carbon atoms (C15, C17, C19, C21) are found, their contents do not exceed 1% and only in gonads content of C19 makes 5%. There are big contents of monoenoic FA of different families in blood (41%). The contribution of eicosapentaenoic PUFA (20:5, 15.5-26.1%) and families of C22 FA (9.3-19.5%), including a docosahexaenoic, PUFA (22:6, 3.0-4.1%) are significant in the tissues investigated. The content of arachidonic PUFA (20:4) is insignificant (traces - 2.2%). In all *U. unicinctus* tissues investigated, the greatest contribution falls at a share of 20:5 PUFA.

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STUDY OF HEMOLYTIC AND ANTIMICROBIAL ACTIVITIES OF THE LOW-MOLECULAR WEIGHT METABOLITES FROM *KALANCHOE DAIGREMONTIANA*

Chaikina, E.L.[1], Gerasimenko, N.I.[1], Serebryakov, Yu.M.[2], Pivkin, M.V., Shtoll, I.R.[2], Anisimov, M.M.[1]

[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*

[2] *Seaside Research Veterinary Station, Vladivostok, Russia*

e-mail: anisimov@piboc.dvo.ru;

For the last decade the apparent tendency to wider application of vegetable pharmaceuticals is observed. It is due to their low toxicity, soft action, practically full absence of allergic reactions, a wide spectrum of pharmacological properties, and influence on many biochemical indexes. One of the plants popular in traditional medicine is *Kalanchoe daigremontiana* (Hamet de la Bathie) Jacobs et Perr. Juice of this plant has antimicrobial activity and is applied for the treatment of a rhinitis, healing of wounds and ulcers. However, it is not quite clear what exactly causes its healing effect?

To reveal biological activity, in particular, antimicrobial and pH-dependent hemolytic activity, a group of substances of various chemical nature was isolated from *K. daigremontiana*. To isolate these substances, the plant (500 g) was triply extracted with 96 % ethanol. A lipid-pigment fraction (1.37 g) was then isolated with chloroform from combined ethanol extract (EE). EE fraction was concentrated to minimum volume and reprecipitated in ethanol to obtain a residue of water-soluble polysaccharide I (0.82 g). After separating polysaccharide, the ethanol solution was used to obtain a complex of polyphenolic compounds (70 mg). The residual plant tissue was then extracted with 1 % soda solution followed by the reprecipitation in ethanol to obtain polysaccharide II (5.7 g). Using column chromatography on silica gel, chlorophylls (22.3), carotenes (8.2), hydrocarbons (9.3), sterines (6), ethers of sterines (3.6), free fatty acids (FFA, 16,7), ethers of fatty acids (7.7), triglycerides (14), monogalactosyldiacylglycerol (MGDG, 3.4), sulfoquinovosyldiacylglycerol (SQDG, 3.7), and phospholipids (4.9) were isolated from the lipid-pigment fraction (% from weight of the fraction).

To determine hemolytic activity, suspension of erythrocytes was incubated at 37 ^{o}C during 180 minutes. The haemolysis was estimated visually by 100% destruction of the erythrocytes. Antimicrobial activity was studied using standard test-cultures: *Safale* S-04, *Candida albicans* KMM 455, *Fusarium oxysperum* KMM 4639, *Aspergillus niger* KMM 4634, *Staphylococcus aureus* ATCC 21027, *Escherichia coli* ATCC 15034). This activity was estimated by the width of an inhibition zone of the growth of microorganisms in agar medium (in mm from the edge of a hole).

The metabolites studied can be divided into three main groups according to their hemolytic activity. The first group includes EE, polyphenol compounds, FFA, and sterines showing pH-dependent properties. The second one includes carotenes having membranothropic activity independent of pH. Polysaccharides I and II, chlorophylls, triglycerides, MGDG, and SQDG belong to the third group of these substances and are proved to be inactive at two pH meanings in the concentration up to 100 μg/ml.

EE, chlorophylls, polysaccharides I and II were found to be rather active against the yeast cells of *Safale* S-04 and *C. albicans*, fungi *F. oxysperum* as well as against bacteria *S. aureus* and *E. coli*. Sterines, ttriglycerides, and SQDG were inactive against all the microorganisms listed above.

Thus, a large group of the substances isolated from the plant *Kalanchoe daigremontiana*, have biological activity and are promising for the further studying.

NEW LECTIN FROM THE RED MARINE ALGA *TICHOCARPUS CRINITUS*

Chernikov, O.V., Molchanova, V.I., Chikalovets, I.V., Lukyanov, P.A.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: chernikol@yandex.ru

Lectins are proteins or glycoproteins, reversibly and selectively interacting with mono- and oligosaccharides, that are widespread in biological world, from viruses to mammals. From the moment of the first detection of lectins in algal species considerable progress has been reported in the study of algal lectins. To date, most of the agglutinins purified from algae have been obtained from red algae. However the number of these lectins purified and characterized in detail is still considered small compared to those for lectins of higher plants and invertebrates. In spite of the progress made in the biochemical characterization of marine algal lectins, additional information is needed for a more comprehensive understanding of their properties, structures and possible biological functions.

A new lectin, named TCL, was isolated from the red alga *Tichocarpus crinitus* by the combination of hydrophobic and gel-penetrating chromatography methods. Gel-filtration and SDS-PAGE in reducing and non-reducing conditions indicate that it exists as 41 kDa protein in its native state. Analysis of its amino acid composition revealed the high content of serine, proline, alanine, acidic and hydroxyl amino acids. The content of acidic amino acids (aspartic and glutamic) was fairly higher than that of basic amino acids (lysine, histidine and arginine). It coincide with the low isoelectric point of TCL (4.93). TCL was found to agglutinate best rabbit erythrocytes. TCL is thermostable, Ca^{2+}-independent lectin with carbohydrate content of 9.7%. This lectin displays the highest activity in the range of pH 7.0-8.0. Hemagglutination activity of this lectin was inhibited by the complex glycoproteins porcine stomach mucin and fetuin, but not by any of the monosaccharides tested.

Lectins have captured the attention of a large number of researchers on account of the various exploitable activities that they exhibit, including their proliferative effects on various cell types. TCL shows a weak mitogenic effect on human T-lymphocytes. The activity was dose-dependent, showing the optimum concentration of 0.78 μg/ml. We observed a gradual reduction in mitogenic capacity with increase in the lectin concentration. The degree of the mitogenic activity of TCL was lower (about 50%) to that of Concanavaline A.

Cytokine production by not stimulated and *E. coli* lipopolysaccharide (LPS) stimulated cells in response to different concentrations of the TCL *in vitro* was investigated also. It has been shown that TCL is multicytokine inductor, ensuring increase of production of pro-inflammatory cytokines (TNF-α, IFN-γ and IL-6). The results display a direct correlation between the cytokines levels in the supernatants from not LPS stimulated cells and lectin concentrations.

Known, that lectins often play an important role in a defense system. It was shown that TCL combined with some microorganisms. Lectin did not inhibit the growth of cultures of microorganism and even stimulated some of them.

Lectins are widely used in biomedical studies as well as for development of diagnostic test-systems, since malignant transformation of cells is known to be accompanied by disordering one of the most important processes in cell – glycosylation. A method of Enzyme-Linked Lectin Assay using lectin TCL was developed. Considerable difference between binding of TCL with blood serum glycoconjugates of cancer patients and healthy persons was revealed.

Thereby, our results suggested that TCL might be a useful tool for biomedical and biochemical purposes.

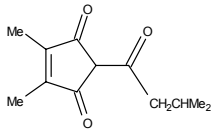
The work was partly supported by the grants from Far Eastern Branch of Russian Academy of Sciences and Presidium of Russian Academy of Sciences “Basic Research in Medicine” (06-III-B-05-134; 06-I-П-12-040).

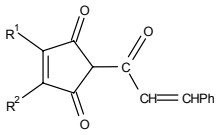
EFFECTS OF NATURAL AND SYNTHETIC 2-ACYLCYCLOPENT-4-ENE-1,3-DIONES AND THEIR DERIVATIVES ON THE ROOT GROWTH OF *FAGOPYRUM ESCULENTUM* SEEDLINGS

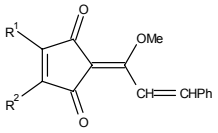
Demina, E.A., Shestak, O.P., Novikov, V.L., Anisimov, M.M.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: dminae@mail.ru

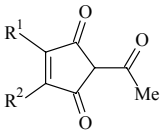
The free cyclopentene *β,β'*-triketones calythrone, linderone, lucidone and coruscanone B, their natural methyl enol ethers methyllinderone, methyllucidone and coruscanone A, and their synthetic analogues attract attention by their high chemical and biological potential. At the same time effect of natural compounds on higher plants, in particular, on seedlings growth, has not been studied. The aim of this work was investigation of the influence of natural and synthetic cyclopentene *β,β'*-triketones, their methyl enol ethers and some derivatives on root growth of *Fagopyrum esculentum* Moench. seedlings.

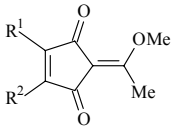


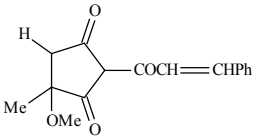


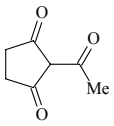


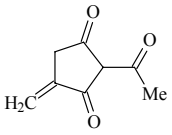
1 Calythrone **2** Linderone R^1=R^2=OMe **5** Methyllinderone R^1=R^2=OMe
3 Lucidone R^1=OMe, R^2=H **6** Methyllucidone R^1=OMe,R^2=H
4 Coruscanone B R^1=Me, R^2=H **7** Coruscanone A R^1=Me, R^2=H











8 R^1=R^2=H; **9** R^1 =Me, R^2= H **15** R^1 =Me, R^2= H **17** **18** **19**
10 R^1= Cl, R^2= H; **11** R^1= Br, R^2= H **16** R^1=R^2=Cl
12 R^1=R^2=Me; **13** R^1= Cl, R^2= Me
14 R^1=R^2=Cl

Of the all compounds investigated, 2-acetyl-4,5-dichlorocyclopent-4-ene-1,3-dione was found to be the most active inhibitor; the saturated 2-acetylcyclopentane-1,3-dione the least active.

The double bond at the position 4(5) is very important for inhibiting effect of the triketones. After removal of this bond from the structure, the activity of compounds is reduced. The shift of this double bond from the cycle to a side chain resulted in significant decrease of activity. Nature of substituents at the positions 4 and 5 of *β,β'*-triketones and their number essentially influence the activity of the investigated substances. Nature of acyl radical at C-2 is very important for effect of the 2-acylcyclopent-4-ene-1,3-diones on root growth of buckwheat seedlings. The conversion of free triketones in methyl enol ethers have different effects on activity of prepared substances. In case of natural compounds, this conversion results in increase of root growth inhibition. But synthetic methyl enol ethers were much more active than free triketones.

The special attention should be paid to the effect of the coruscanone B and 2-acetylcyclopent-4-ene-1,3-dione which, in the concentration of 10.0 μg/ml or above, act as inhibitors of root growth of *F. esculentum* seedlings, while in the concentration of 0.01 -1.0 μg/ml they significantly promote the root growth.

Thus, it has been shown that natural cyclopentene *β,β'*-triketones and their methyl enol ethers are *F. esculentum* seedlings growth regulators. It has been demonstrated that chemical structures of cyclopentene *β,β'*-triketones are very important for their biological effects.

AAPTAMINES - THE ANTICANCER SUBSTANCES FROM MARINE SPONGE *AAPTOS SP.*

[1]Dyshlovoy, S.A., [2]Fedorov, S.N., [2]Shubina, L.K., [2]Kalinovsky, A.I., [2]Savina, A.S., [2]Stonik, V.A.

[1]Far-Eastern National University, Vladivostok.

[2] Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia

e-mail: dishlovoy@mail.ru

Marine sponges are a rich source of the natural compounds possessing potent biological activities and belonging to various structural groups.

The bioactivity-guided fractionation has allowed us to isolate three substances possessing anticancer properties from the ethanol-aqueous extract of the marine sponge *Aaptos sp*., collected near the coast of Vietnam. The isolated substances belong to the aaptamine structural group. Among them there are two previously known alkaloids aaptamine (**1)** and 9-demethyloxyaaptamine (**2**), and a new alkaloid 3-(N-morpholyle)-9-demethyloxyaaptamine (**3**) (**Figure**).

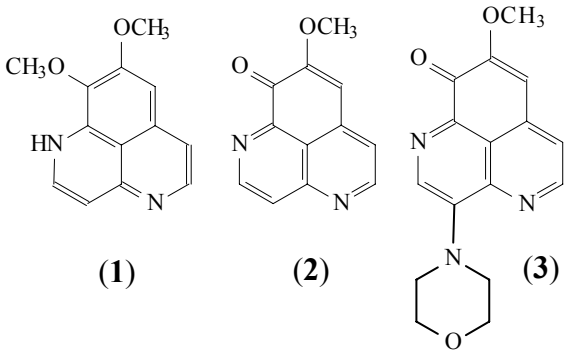


Figure. Three aaptamine alkaloids from the marine sponge *Aaptos* sp.

Anticancer properties of the isolated substances **1-3** were further studied using the MTS-method for cytotoxicity evaluation and the soft agar method for the the cancer-preventive activity. Unfortunately, it was impossible to use the MTS-method for evaluation of IC_{50} of compound (**3**) against some cell lines due to intensive red color solutions at concentrations of 50 μM and more.

The results showed that the substances **1-3** are most active against HL-60 promyelocytic leukemia cell line among all cancer cell lines studied.

The significant resistance of JB6 P^+ Cl41 Ras DNM cells to the cytotoxic action of (**1**) and (**2**) in comparison with JB6 P^+ Cl41 cells showed that Ras signaling pathway may play a crucial role in aaptamine-like substances–induced cytotoxicity, and therefore these compounds may be used as tools for the study of the Ras-protein role in carcinogenesis.

Here in we report that compounds (**1**) and (**2**) inhibited p53-dependent transcriptional activity in cytotoxic concentrations. This important finding demonstrated that cytotoxicity induced by these compounds is p53-independent and therefore these substances may be used for the treatment of the p53-deficient cancer cell lines (such as HL-60), which are resistant to a number of medicines.

The soft-agar method of evaluation of the cancer-preventive properties demonstrated that compounds (**1**) and (**2**) inhibited the growth of the malignantly transformed JB6 P^+ Cl 41 cells in concentrations 4-20 times less then cytotoxic concentrations ($INCC_{50}$ = 2 μM and $INCC_{50}$ = 0.75 μM respectively), indicated that aaptamine-like compounds can be perspective as cancer-preventive drugs.

LIPOPOLYSACCHARIDE ASSEMBLING IN THE LIPID TERMINAL OF ENVIRONMENT BACTERIA; RECOVERY OF THE ENSEMBLE HETEROGENEITY BY VIBRATIONAL COOLING FOURIER TRANSFORM MASS SPECTROMETRY

Elkin Yu.N.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: yurielkin@piboc.dvo.ru

A phenomenon of the "intrinsic" heterogeneity of the lipid terminal (LT) in the lipopolysaccharide (LPS) of pathogenic and environmental bacteria displayed by mass spectrometry as a paint brush-like profile was not the item paid by an adequate attention. The phenomenon surmises the LPS molecules that carry a wide variety of LTs. The molecular design of the lipid terminal in the intact LPS of a marine bacterium *Pseudoalteromonas haloplanktis* ATCC 14393^T was deciphered basing on mass spectrometry techniques: ESI and VC MALDI FTMS/MS [IRMPD and SORI CAD, respectively], nESI QMS, TLC [off-line and on-line] MALDI TOFMS and VC MALDI FTMS, respectively. The lipid isolated from the LPS showed an "intrinsic" heterogeneity in each of six ion clusters differing by number of acyl and phosphate groups. Four residues of different hydroxy fatty acids [10:0; 11:0(i-10:0); 12:0; 13:0(i-12:0)] were revealed instead of the typical single acyl group (*e.g.* 3OH14:0 acid in *E.coli*) in many other bacteria. The enigmatic profile for the lipid has appeared to be a result of an enzyme convolution due to involving a pool of ACP-acyl precursors. Triple permutations of the four-member precursor pool led to an "intrinsic" heterogeneity pattern of molecular adduct ions for a homogeneous lipid species which exists as a lipid ensemble. Corporative lipid molecules of the ensemble differed by the total primary acyl chain length up to 9 carbons that was an obstacle to detect them by both mass spectrometry ionization techniques without distortion of the ensemble pattern. Partial de-O-acylation and de-phosphorylation during isolation of the lipid terminal additionally masked it a native image. The vibrational cooling MALDI FTMS SORI CAD technique using the silica gel target was found to be the indispensable method to recognize three species of the lipid ensembles totally composed of 27 molecular types at least. Two **a**- and **b**-species of the ensembles have the common structural framework of the 1,4'-bi-phosphorylated di-glucosamine acylated with a pool of hydroxy acyl groups in positions 2, 2' and 3'. The remaining primary position 3 is acylated with the single 3OH11:0 acid residue. Fifth secondary acyl group (the pool of 12:0 and 12:1 acids) giving rise **a**- and **b**-species, respectively, rides 3-OH acyl groups in position 3'. Third minor **c**-species of the ensembles is the tetra-acyl lipid ensemble that putatively carries a single 12:0 acid residue in position 3' instead of the pool of acyloxyacyl groups.

ANTICANCER ACTIVITY OF FUCOIDAN FROM THE BROWN SEAWEED *LAMINARIA GURIANOVAE*.

Ermakova, S.P., Lee, N.Y., Kang, K.W., Vischuk, O.C., Kusaykin, M.I., Zvyagintseva, T.N., Hong Seok Choi

[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*

[2] *College of Pharmacy, Chosun University, Gwangju, 501-759, South Korea*

e-mail:swetlana_e@mail.ru

Brown seaweeds represent a rich and easily regenerated source of polysaccharides of structural and biological activity interest: laminarans, fucoidans, uronans, and also alginic acids. Fucoidans are the family of sulphated homo-and heteropolysaccharides mainly build up of alpha-L-fucose with sulphate and acetate units. Fucoidans have anti-neoplastic, anti-coagulant, anti-complementary, anti-virus activities, including anti-HIV infection, herpes viruses. It was recently reported that fucoidan increases the level of nitric oxide (NO) production which was related with p38 kinase-dependent NF-kB activation. Although several studies on the biological activities of fucoidans have been performed, with particular focus on its antitumorigenic activity, it is unclear if fucoidan inhibits the neoplastic cell transformation and AP-1 transactivation activity induced by tumor promoter, such as EGF. Fucoidan from *Laminaria gurjanovae* differs from similar polysaccharides, isolated from other *Laminaria* species, which are sulfated poly-(1→3)-alpha-*L*-fucans. An increased content of galactose was described for fucoidans from *L. japonica* and *Ecklonia kurome*. This fucoidan is a partially acetylated galactofucan of a block structure, in which both monosaccharide residues are sulfated. The JB6 Cl41 cell line is a well-established system used extensively as an in vitro model for the study of tumor promotion and antitumor promotion.

To elucidate the mechanism of the anti-tumorigenic effects of fucoidan, we studied the effects of fucoidan extracted from *L. guryanovae* on the phosphorylation of EGFR and neoplastic cell transformation induced by EGF in mouse epidermal JB6 cells. Neoplastic transformation is often associated with a dramatic increase in AP-1 activity, and this transient induction of AP-1 has been shown to be involved in the promotion of epidermal tumors. Constitutive AP-1 activity has been associated with the malignant conversion of papillomas to carcinomas as well.

The epidermal growth factor receptor (EGFR), one of the receptor tyrosine kinases, plays a pivotal role in regulating cell proliferation, differentiation, and transformation. The EGFR is an important target for cancer therapy. Many carcinomas are promoted by EGFR activation, which can result from mutation of the receptor, its overexpression or from EGFR stimulation through autocrine loops. Because the activation of EGFR has an important role in tumorigenesis, the results of this investigation may provide new insights in the mechanism of fucoidan action in tumor suppression and the possibility for its application in tumor prevention and treatment.

In our experiments, fucoidan from *L. gurjanovae* suppressed EGF-stimulated JB6 Cl41 cell transformation on soft agar. A corresponding inhibition of EGF-induced AP-1 activity was also found, suggesting that the inhibition of tumorigenesis by this polysaccharide is through the inhibition of AP-1 activity. Many mechanisms are involved in the up- and down-regulation of AP-1 activity. MAPKs are the most common pathways known to mediate AP-1 function, and in the current experiment, EGF activated members of the MAPK family: JNK, ERKs.

Our results indicated that fucoidan from *L. gurjanovae*, blocked EGF-induced ERK and JNK activity, which most likely explains their inhibitory effect on AP-1 transactivation in cell transformation induced by EGF. Moreover, EGF-induced the *c-fos* and *c-jun* transcriptional activities were inhibited by fucoidan, resulting to the suppression of AP-1 activity and cell transformation induced by EGF. Taken together, these results indicate that fucoidan might exert chemopreventive effects through the inhibition of phosphorylation of the EGFR.

SERINE PROTEINASES INHIBITORS FROM THE SEA ANEMONE *RADIANTHUS MACRODACTYLUS*

Gladkikh, I.N., Leuchenko, E.V., Monastyrnaya, M.M., Chausova, V.N., Kozlovskaya, E.P.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: Sokotun@piboc.dvo.ru

Searching of biological active nature compounds which specifically interact with proteolytic enzymes and regulate their activity is one of the most interesting and important problem of bioorganic chemistry. Proteinases play a key role in protein metabolism of different organisms. It is known that basic level of regulation of enzymes action is an inhibition of their proteolytic activity. In this connection with the investigation of structure functional features, specificity and the mechanism of action of the proteinase inhibitors is the fundamental problem which elucidates the molecular basics of protein-protein interactions. These studies can be serving as a basis for development of therapeutic agents for the treatment of diseases caused by derangement of physical regulation of activity of proteolytic enzymes.

There are many publications devoted to the study of structural and functional activity of serine proteinase inhibitors isolated from various plants and animals [1-3]. In the same time much less is known about the proteinase inhibitors from marine organisms, toxic coelenterate, anemones. More then twenty years it is known that inhibitors are produced by sea anemones with neurotoxins and cytotoxins, but up to now the convincing explanation of this phenomenon is absent. It was established that some proteinase inhibitors from sea anemones have the ability to block potential-dependent potassium channels and to interact with a vanilloid receptor type TRPV1, demonstrating antihistaminic activity. This information indicates on their polyfunctional activity of these compounds. [1, 4-5].

Three new serine proteinase inhibitors InhVJ, RmIn I и RmIn II from the tropical sea anemone *Radiantus macrodactylus* were isolated and characterized. The polypeptides were assigned to the family of the Kunitz inhibitor. The amino acid sequences of N-terminal fragments of RmIn I и RmIn II and complete sequence of InhVJ were determined. Nucleotide sequence of cDNA encoding mature inhibitor was determined by the methods of molecular cloning. The amino acid sequences of InhVJ, RmIn I и RmIn II were found to be very similar to the corresponding sequences of inhibitors from coelenterate, reptile and mammals. It was shown that the replacement of only one amino acid in a reactive centre of inhibitors reduce its inhibiting activity.

The spatial organization of the inhibitor InhVJ at the levels of secondary and tertiary structure was studied by the methods of the UV- and CD spectroscopy. It was shown that secondary structure of InhVJ is highly ordered, the tertiary structure is rigid and can be related to mix α+β polypeptides.

The specificity of isolated inhibitors and its interaction with different proteases were investigated. Inhibitors InhVJ, RmIn I и RmIn II are highly specific polypeptides because they inhibit only two serine proteinase activities: trypsin and chymotrypsin. It was established that the reaction of the inhibitor with both proteinases follows a 1:1 molar stoichiometry. Kinetic and thermodynamic parameters of the complexes inhibitor-proteinase were determined.

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[3] Krowarsch D., Cierpicki T., Jelen F., Otlewski J. Canonical protein inhibitors of serine proteases. *Cell. Mol. Life Science*. 2003, **60**, 2427-2444.
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[5] Honma T., Kawahata S., Ishida M., Nagai H., Nagashima Y., Shiomi K. Novel peptide toxins from the sea anemone *Stichodactyla haddoni*. *Peptides.* 2008. **29**, 536-544.

DFT STUDY OF THE STRUCTURE AND ANTIOXIDANT PROPERTIES OF DISODIUM SALTS OF 2,3,5,6,8-PENTAHYDROXY-7-ETHYL-1,4-NAPHTHOQUINONE (ECHINOCHROME A)

Glazunov, V.P., Berdyshev, D.V., Novikov, V.L.

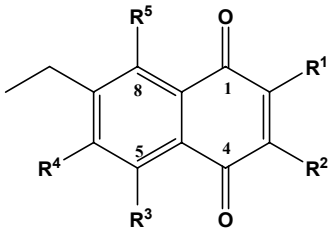
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
E-mail: *glazunov@piboc.dvo.ru*

The mixture of disodium salts of echinochrome A (ECHA, **1**), the pigment of sea urchins, forms the basis for the pharmacopeial drug «Histochrome®» used in cardiology and ophthalmology. To understand the fine mechanism of Histochrome's action *in vivo* it is important to know the structure and physicochemical properties of these salts. Since up to present these characteristics of sodium salts have remained unknown we have studied the structure of salts **2** - **11** and their radicals **12** - **26**, obtained by the reaction with hydroperoxyl radical, by using the density functional theory.

Unfortunately, the experimental investigations give no way for solving a problem about an acidity either of the five OH groups of ECHA. What hydroxyl group in this compound has a maximum acidity? Thus the evaluation of the relative gas-phase acidities of these OH groups was carried out via calculation of the heterolytic dissociation energies of O–H bonds for all OH groups of molecule **1** by the use of B3LYP/6-311G(d) and B3LYP/6-311G(d,p) methods. It was shown that 2β- and 6β-OH groups are the most acidic OH groups in molecule **1**. Their acidity exceed the one of acetic acid. The surprising thing is that the acidity of 5α-OH group is higher then one of 3β-OH group.

Based on the results of the Gibbs energies and the enthalpies calculations using the (U)B3LYP/6-31G(d) and (U)B3LYP/6-311G(d) methods, we carried out the conformational analysis of disodium salts **2** – **11**, obtained by the reaction of molecule **1** with NaOH, and their radicals **12** – **26** with allowance for tautomerism involving the α-OH groups and rotational isomerism involving the β-OH groups (as a result of internal rotation of three β-OH groups about corresponding C–O bonds). The primary screening of all tautomers and rotamers of compounds **2** – **26** by the (U)B3LYP/6-31G(d) was followed by full optimization of the geometries of the isomers characterized by the percentages of about 1% and higher by the (U)B3LYP/6-311G(d) method. Only these isomers of compounds **2** – **26** were taken in account in considering the energy balances of the reaction with hydroperoxyl radical.

The heats of the reactions of molecule **1** with sodium hydroxide were evaluated by the B3LYP/6-311(d) method.

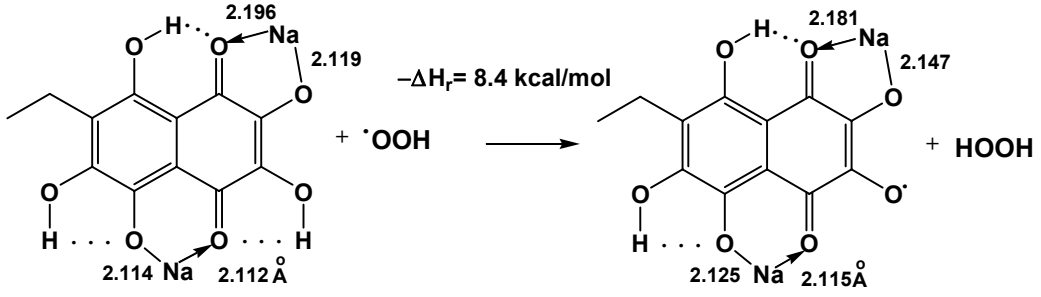


Sample	R^1	R^2	R^3	R^4	R^5
1	OH	OH	OH	OH	OH
2	ONa	ONa	OH	OH	OH
3	ONa	OH	ONa	OH	OH
4	ONa	OH	OH	ONa	OH
5	ONa	OH	OH	OH	ONa
6	OH	ONa	ONa	OH	OH
7	OH	ONa	OH	ONa	OH
8	OH	ONa	OH	OH	ONa
9	OH	OH	ONa	ONa	OH
10	OH	OH	ONa	OH	ONa
11	OH	OH	OH	ONa	ONa
12*	ONa	ONa	O$^{\bullet}$	OH	OH
13*	ONa	ONa	OH	O$^{\bullet}$	OH
14*	ONa	ONa	OH	OH	O$^{\bullet}$
15	ONa	O$^{\bullet}$	ONa	OH	OH
16	ONa	OH	ONa	O$^{\bullet}$	OH
17	ONa	OH	ONa	OH	O$^{\bullet}$
18	ONa	O$^{\bullet}$	OH	ONa	OH
19	ONa	OH	O$^{\bullet}$	ONa	OH
20	ONa	OH	OH	ONa	O$^{\bullet}$
21*	ONa	O$^{\bullet}$	OH	OH	ONa
22*	ONa	OH	O$^{\bullet}$	OH	ONa
23*	ONa	OH	OH	O$^{\bullet}$	ONa
24	O$^{\bullet}$	OH	ONa	OH	ONa
25	OH	O$^{\bullet}$	ONa	OH	ONa
26	OH	OH	ONa	O$^{\bullet}$	ONa

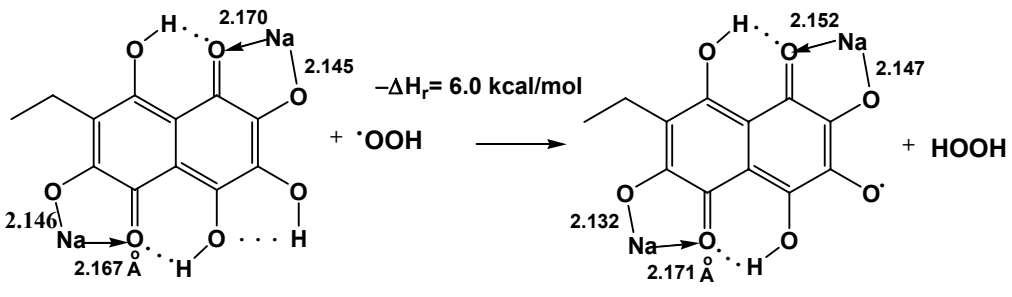
*- do not calculated because content of **2** and **5** is less than < 10^{-7}%.

It was shown that among disodium salts **2** – **11** only **3A**, **4D** and **10** are realized to a marked extent. The content of these compounds to the total amount of ECHA's hypothetically где сказуемое possible disodium salts is 99.7%. The content of salts **3A** and **10** obtaining by the neutralization of 2β-OH-, 5α-OH- and 5α-OH-, 8α-OH-groups, respectively, may be more then 50%.

It has been found that the gas-phase reactions of hydroperoxyl radical quenching by disodium salts of ECHA are exothermic. Among all the theoretically possible radicals **12** – **26** the energetically most favorable are radicals **15A** and **18D.** The radical **15A** obtaining by the quenching of hydroperoxyl radical with disodium salt **3A** is dominat. Its content is about 98%.



3A **15A**



4D **18D**

IN VITRO RESEARCH OF BIOLOGICAL ACTIVITY OF ZEOLITES OF SOME FAR-EASTERN DEPOSITS

Golokhvast, K.S.[1], Anisimova, A.A.[2]

[1] *Institute of oil and gas Far Eastern State Technical University, Vladivostok, Russia*
[2] *Far-Eastern State Technical University, Vladivostok, Russia*
e-mail: droopy@mail.ru

The series of cytological, biotechnological and microbiological experiments we propose will let to determine some biological properties of zeolites *in vitro*, in animal and human cell culture as well as in microorganism one. In the present work the zeolites of different deposits of Russian Far East will be researched.

To evaluate of cytotoxic properties of zeolites and their antitumour effect the follow cell lines will be taken: HT-29 (intestinal cancer), JB6 Cl41, mesenchymal stem cells, alveolar macrophages and lymphocytes. Cytotoxic effect will be studied by the MTS-method and by means of calculation of cell colonies number. To estimate of cell malignisation the method of cultivation in soft agar will be used.

To evaluate of probiotic effect of zeolites the cell cultures of *Lactobacillus acidophilus, Bifidobacterium bifidum, Bacillus subtilis* and other microorganisms of native intestinal flora of human and animals will be studied with the standard methods.

We have already got some results concerning biological properties of zeolytes. When getting into a rat lung by inhalation the zeolites of two deposits, Vanginskoe and Kulikovskoe, possess a ctyoprotect effect on the local immune system of lung as well as general antioxidant effect. Besides the zeolites prove themselves as immunomodulators altering representatively the proportion between alveolar macrophages and lymphocytes *in vivo* (Golokhvast et al., 2005—2008). Taking into account a need of regular antigen stimulation of immune system it could be supposed that inhalation of mineral dust is necessary to a normal lung immunity functioning (because of sterility of alveoli in norm) and is of great evolutionary importance. Reproduction of these experiments *in vitro* will let to reveal the cell and molecular mechanisms of biological activity of zeolites and to explain the pathogenesis of some diseases of bronchopulmonary system which undergoes a pressure of such factor as mineral dust every day.

We have carried out preliminary study of cytotoxic properties of zeolites *in vitro*. The zeolites of the deposits Kulikovskoe and Lyulyinskoe don't display any toxic effect on the normal skin cells of mouse (the cell line JB6 Cl41) when are taken in concentration of 0.01, 0.1 и 1 mg/ml. Also these zeolites don't inhibit representatively a cell colonies growth of the intestinal cancer culture (the cell line HT-29) in soft agar when are taken in nontoxic concentrations (0.01, 0.1 и 1 mg/ml). The subsequent experiments in this direction are required.

CHLOROTOPSENTIASTEROL SULFATE D, THE FIRST CHLOROFURAN-CONTAINING STEROID SULFATE, AND TOPSENTIASTEROL SULFATE F FROM A MARINE SPONGE *TOPSENTIA* SP

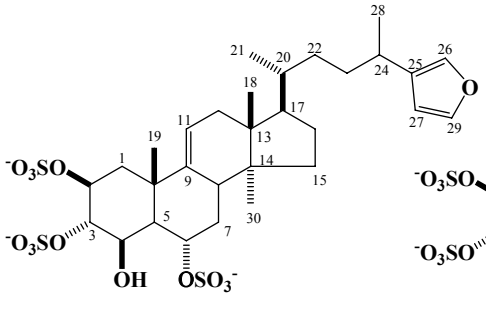
Guzii, A.G., Makarieva, T. N., Denisenko, V. A., Dmitrenok, P. S., Burtseva, Y.V., Krasokhin, V.B., Stonik, V. A.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia

e-mail: makarieva@piboc.dvo.ru

Topsentiasterol sulfates are a small group of sulfated polyhydroxysteroids from sponges of the genus *Topsentia* (family Halichondriidae).[1] All these steroid polysulfates have $\Delta^{9(11)}$ steroid nucleus with methylation at C14, sulfate groups at 2β, 3α, 6α positions and hydroxyl at C4. Early findings on biological properties of topsentiasterol sulfates included antibacterial and antifungal activities.[1,2] It has been demonstrated that related trihydroxysteroid sulfates influence on activity of 1,3-β-glucanases.[3]

In the course of our continuing studies on marine natural products, we have isolated from a marine sponge *Topsentia* sp the known topsentiasterol sulfate D (**1**) and two new compounds named as chlorotopsentiasterol sulfate D (**2**) and topsentiasterol sulfate F (**3**) .



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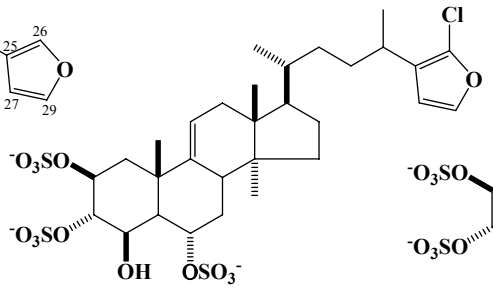

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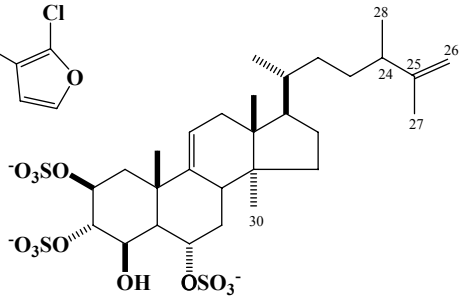
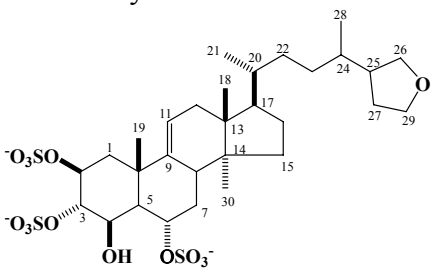


3

Structural elucidation of **1-3** has been carried out using spectroscopic methods including COSY, DEPT, HSQC and HMBC data, chemical correlations and HRESIMS. From high-resolution negative ESIMS, the molecular formula of **2** was established as $C_{30}H_{44}O_{14}S_3Cl$ (*m/z* 759.1575 $[M_{3H}–H]^-$; *Δ* 0.9 ppm). These molecules also showed MS isotopic patterns characteristic of monochlorinated secondary metabolites ($[M_{3H}–H]^-$: $[M_{3H}–H+2]^-$ = 3:1. Compounds **1** and **2** were hydrogenated over PtO_2 to give the same compound (**4**) which was identified by NMR and ESIMS data.



4

Chlorotopsentiasterol sulfate D (**2**) contains a rare chlorofuran fragment that has not been previously found in natural products. Chlorotopsentiasterol sulfate D and topsentiasterol sulfates D, and F exhibit endo-1,3-β-D-glucanase *Spisula sachalinensis* activity with a I_{50} of 2.2, 4.77, and 18.60 μg/mL, respectively.

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STRUCTURE AND BIOSYNTHESIS OF CARRAGEENAN: THE MAIN COMPONENT OF RED ALGAL CELL WALL STRUCTURES

Helbert W., Genicot S., Correc G., Jouaneau D.
Marine Plant and Biomolecules, University Piere and Marie Curie-CNRS (UMR 7139), Station Biologique, Roscoff, France
e-mail: helbert@sb-roscoff.fr

Carrageenans are sulphated galactans which unique rheological and texturizing properties are widely exploited in laboratory and in industry. These molecules are the main components of red algal (Rhodophyta) cell wall which concentration reaches up to 50% of the dry mass of the seaweed. As a consequence, carrageenans are densely packed in the cell wall under the form of a tri-dimensional solid network of aggregated fibbers. This large family of hydrocolloids is made up of linear chains of galactose, with alternating α(1→3) and β(1→4) linkages. Carrageenans are classified according to the number and the position of sulphated ester (S), and by the occurrence of 3,6 anhydro-bridges in the α-linked residues (DA-unit) found in gelling carrageenans. For example, the three most industrially exploited carrageenans, namely kappa- (κ, DA-G4S), iota- (ι, DA2S-G4S) and lambda- (λ, D2S6S-G2S) carrageenans, are distinguished by the presence of one, two and three esters sulfate groups per repeating disaccharide unit, respectively. However, carrageenans have very heterogeneous chemical structures, depending on algal source, life stage and extraction procedure. This structural complexity is attributed to the occurrence of a mixture of carrageenans in extracts as well as to the combination of ideal carrabiose motives in purified carrageenans giving rise to hybrids or copolymer chains.

In order to decipher the complex structure of carrageenans and their biosynthetic pathways, we have implemented a strategy allowing the structural characterization of these hybrid macromolecules. This approach is based on the fine structural analysis of enzymes (i.e. carrageenases) degradation products by NMR and mass spectrometry. Notably, we have highlighted various distribution modalities of carrageenan moieties along carrageenan chain. We have also searched for enzymes involved in the biosynthesis of carrageenans. We have purified and investigated two galactose-6-sulfurylases which catalyze the formation of the 3,6 anhydro bridges. We found that these enzymes allow preparing gels that should mimic the physico-chemical properties carrageenan in red algal cell wall.

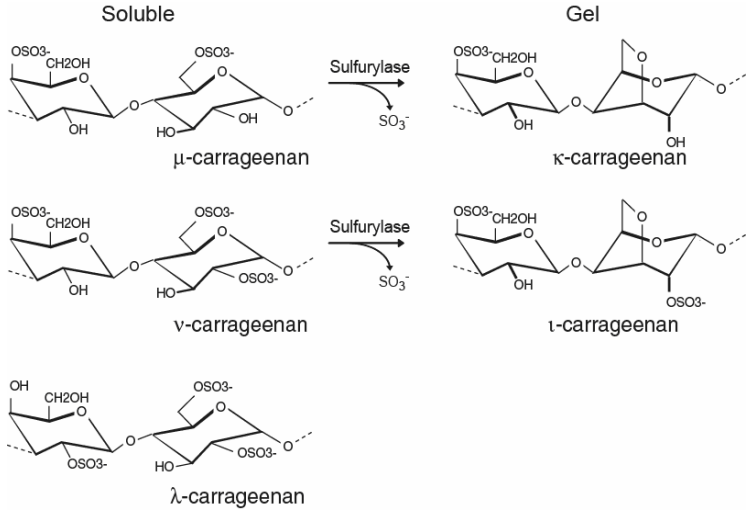


Figure. Chemical structure of carrageenan.

STUDIES ON CHEMICAL CONSTITUENTS OF VIETNAMESE STARFISH *ANTHENEA PENTAGONALA*

Trinh Thi Thu Huong[1], Nguyen Quyet Chien[2], Pham Quoc Long[1]

[1] *Institute of Natural Products Chemistry, VAST-18 Hoang Quoc Viet, Hanoi Vietnam*

[2] *Institute of Chemistry, VAST-18 Hoang Quoc Viet, Hanoi Vietnam*

A large number of studies on the isolation and structure elucidation of glycosphingolipids and saponins from starfish have been reported. This compounds are predominant metabolites of starfish and include a broad variety of biological activities. In the hope of discovering new medicinal resources from marine natural products, we also carried out studying on starfish from Vietnamese sea. In this paper, we report the isolation and structural elucidation from the *n*-hexan extract of the Vietnamese starfish *Anthenea pentagonala*. The component of faty acids and chemical structure of coumpounds 5α-cholestan-3α-ol, 5α-cholest-7-en-3β-ol and a cerebroside "molecular species" has been identified by various spectroscopic means such as GC-MS, EI-MS, ^{1}H-NMR, ^{13}C-NMR, DEPT, HSQC, HMBC, ^{1}H-COSY and by comparision of the spectral data reported earlier. This is the first report of these compounds from the Vietnamese starfish *Anthenea pentagonala*.

MOLECULAR ORGANIZATION AND GENETIC POLYMORPHISM OF NON-SPECIFIC PORIN GENES OF *YERSINIA PSEUDOTUBERCULOSIS*

Isaeva, M.P., Stenkova, A.M., Belyakova, E.P., Guzev, K.V., [2]Shubin, F.N., [3]Rakin, A.V., Rasskazov, V.A.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia

2. RIEM SBRAMS, Selskaya street, 1, Vladivostok,

3 Max von Pettenkofer-Institut, LMU, Pettenkoferstr. 9a, 80336 Munich, Germany

e-mail: issaeva@piboc.dvo.ru

Yersinia pseudotuberculosis is a facultative anaerobe and a psychrotolerant pathogen. It can grow in a variety of ambient conditions such as mammalian organism and environment, therefore, must be able to adapt its behavior to the external different changes. One way of bacterial respond is to regulate membrane permeability.

Porins are outer-membrane proteins which govern the exchanges between gram-negative bacteria and their environment allowing of small hydrophilic molecular diffusion. There are four general porins, named as OmpF, OmpC, PhoE, and OmpN. They account for approximately 2% of the total protein content of the cell and up to 70% of the outer-membrane proteins.

Analysis of Yersinia genomes reveals the four porin protein genes. Sequencing of the 50 *Y. pseudotuberculosis* strains isolated from different geographic area shows the next important findings. One of the porin gene is *ompF* flanked by *aspC*, the aspartate aminotransferase gene and by *asnS*, the asparaginyl-tRNA synthetase gene. All investigated *Y. pseudotuberculosis* strains show a conserved gene arrangement surrounding the porin protein gene *ompF*. An important signature of *ompF* is the 100 % identity of its upstream and downstream sequences. A comparison of protein-coding region of *ompF* reveals 93-100 % conservation between the *Y. pseudotuberculosis* strains. Another porin gene is *ompC* which encodes a generalized OmpC with smaller pore. It has a conserved protein-coding region with 99-100% identity. The promotor sequence displays the major signatures of upstream regulatory sequences *ompC* conserved in species closely related to *E. coli*. A gene arrangement surrounding *ompC* in *Y. pseudotuberculosis* reveals presence of a rearrangement downstream of *ompC* gene in the Far-East strains. Instead of permease they show a gene concern to the transcriptional regulator TetR family. Another gene surrounding ompC gene is *ampH*, the putative penicillin-binding protein gene, conserved in the *Y. pseudotuberculosis* strains. The third porin gene is ompN which encodes minor porin with unknown function in bacteria. Interestingly, this gene reveals a conserved protein-coding region, the same as and the ompC ones. It is uncertain whether *ompN* is an essential gene in other species. Our knowledge of the molecular organization and genetic polymorphism of non-specific porin genes might give us understanding how *Y. pseudotuberculosis* specie may have evolved to help the organism thrive in its environment.

ANTI-TUMOR AND IMMUNO-MODULATING EFFECTS OF FUCOIDAN

Jun-O Jin[1,2], Hae-Young Park[1,2], Qi Xu[1,2], Joo-In Park[1,2], Tatyana Zvyagintseva[3], Valentin A. Stonik[3], and Jong-Young Kwak[1,2]

[1]Department of Biochemistry, School of Medicine and [2]Medical Research Center for Cancer Molecular Therapy, Dong-A University, Busan 602-714, Korea, [3]Pacific Institute of Bioorganic Chemistry, *Far East Branch of the Russian Academy of Sciences, Vladivostok* 690022, Russia

Fucoidan is a natural sulfated polysaccharide (poly-L-fucopyranose) that is isolated from brown algae and has various biological activities. Dendritic cells (DCs) are the most potent antigen-presenting cells for naïve T cells. We evaluated whether human blood DCs isolated from blood *ex vivo* as well as differentiated DCs from monocytes grown in culture could be induced to mature in response to fucoidan. Treatment of these DCs with fucoidan induced maturation of $CD1a^{+}$ MDDCs and the $CD11c^{+}CD123^{low}$ myeloid subset of PBDCs. Interferon-γ secretion and T cell proliferation were enhanced by co-culture of T cells with fucoidan-matured monocytes-derived DCs and peripheral blood DCs. In mouse model, the migration of bone marrow-derived DCs to the spleen was enhanced by fucoidan injection compared to other pattern recognition receptor agonists. Moreover, in murine CT26 colon cancer model, i.v. injection of bone marrow-derived DCs and fucoidan significantly inhibited tumor growth and cured established tumors. These results suggest that fucoidan plays an anti-tumor effect through immuno-modualtion of DCs.

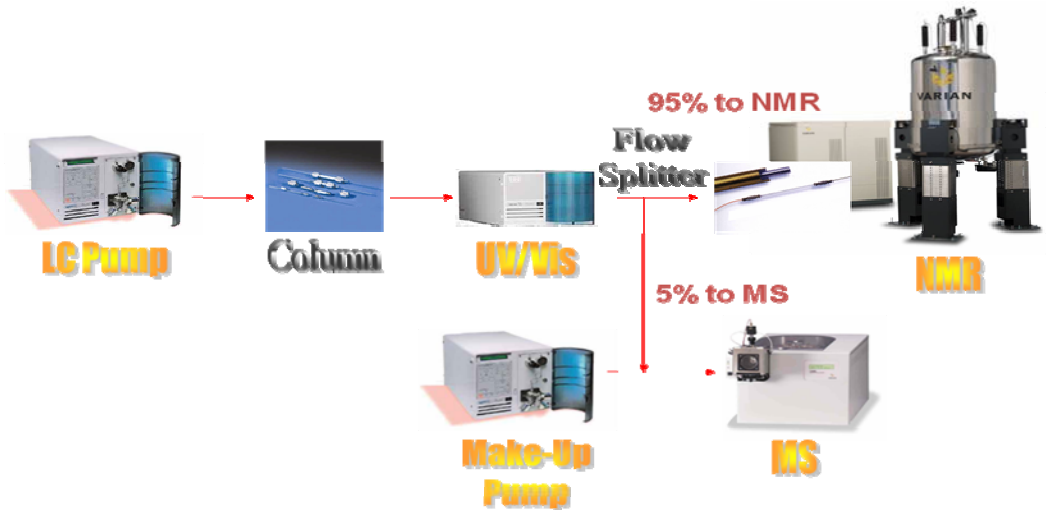
LC-NMR-MS ON NATURAL PRODUCTS RESEARCH

Suk Woo Kang, Byung Hun Um
Korea Institute of Science and Technology (KIST) Gangneung Institute, Gangneung 210-340, Korea (Fax: 0082-650-7299, albertum@kist.re.kr*)*

The hyphenated techniques of LC-NMR and LC-MS are increasingly used in phytochemical analysis. These techniques combine the separation power of HPLC with structural information contents of NMR and MS. The major advantages over the traditional off-line techniques are the rapidity and efficiency. LC-NMR/MS has recently become a valuable tool in analyzing complex mixtures of natural products as well as pharmaceutical metabolites.

The fast identification of isoflavonoids from *Belamcanda chinensis* by LC–NMR and LC–MS: *Belanconda chinesis* is a perennial shrub growing on the hillsides in the East Asia including the Korean peninsula and has been used as Chinese traditional medicine for the treatment of throat ailment such as asthma and tonsillitis. A number of studies about constituents present in *B. chinesis* have been previously reported. However, the separation and purification was necessary for the NMR detection. This study is aimed at identifying major constituents present in *B. chinesis* more rapidly and efficiently. We report the application of the combined use of LC/NMR and LC/MS for separation and structure elucidation of the five isoflavonoids present in *B. chinesis*.

The rapid identification of furanocoumarins from *Angelica dahurica* by LC–NMR/MS
The application of hyphenated LC-NMR/MS has been studied for the direct identification of the major constituents present in *Angelica dahurica*. Reversed-phase isocratic chromatography was performed using acetonitrile-water solvent system on a C_{30} column. NMR and MS spectra for five main peaks were analyzed, which were identified as byakangelicol **(1)**, oxypeucedanin **(2)**, imperatorin **(3)**, phellopterin **(4)**, isoimperatorin **(5)**. This study shows that a combination of LC-NMR/MS techniques can be used for the rapid (40 min) identification of these furanocoumarins



ISOLATION AND FAST IDENTIFICATION OF FLAVONOIDS IN SOPHORA RADIX BY USING PREP-HPLC AND LC-NMR/MS

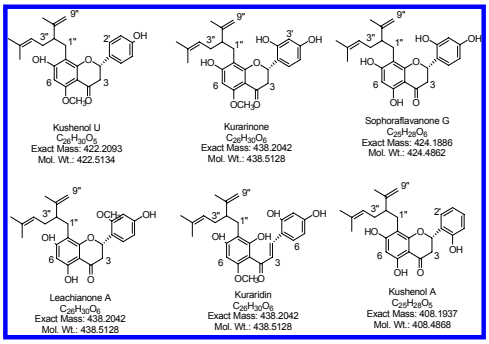
Su Jin Kim, Min Cheol Kim, Suk Woo Kang, Byung Hun Um
Korea Institute of Science and Technology (KIST) Gangneung Institute, Gangneung 210-340, Korea (Fax: 0082-650-7299, albertum@kist.re.kr*)*

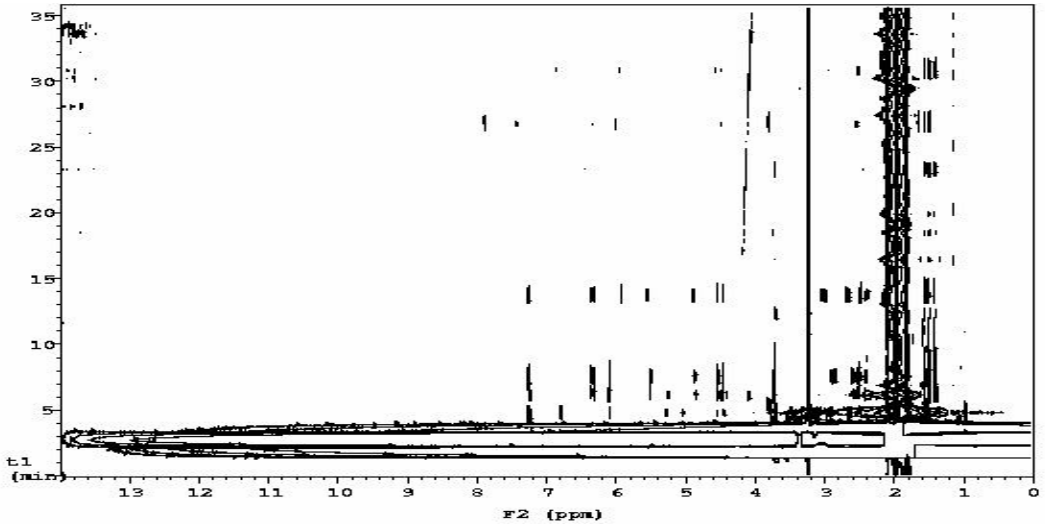
The application of LC-NMR/MS and HPLC on-line ABTS antioxidant screening system for the direct identification of Prenylflavonoids in *Sophora flavescens* has been studied. Prenylflavonoids are major components of Sophora Radix (the roots of *S. flavescens*), a famous oriental medicine. The dried roots of *S. flavescens* were extracted twice with 50ml Ethanol (99.9%) in an ultrasonic bath for an hour and filtered. After evaporating, the ethanolic extract was dissolved in methanol (50mg/ml) and filtered in a 0.45 μm membrane filter. 20 μl of this solution was directly injected into the LC–NMR/MS system using a solvent gradient from 45–65% acetonitrile in D2O for 60 min at a flow-rate of 0.8 ml/min. Separations were monitored by absorbance detection at 320 nm.

The antioxidant active prenylflavonoids were isolated by preparative HPLC and at the same time its antioxidant activities were acessed by preparative HPLC on-line coupled ABTS antioxidant screening system.

By the analysis of the NMR/MS spectrum from on flow LC-NMR/MS, 6 prenyl flavonoids were identified. 1) Kushenol U, 2) Kurarinone, 3) Sophoraflavanone G, 4) Leachianone A, 5) Kuraridin 6) Kushenol A.

Key words: On flow LC-NMR/MS; Antioxidants; Prenylflavonoids; *Sophora flavescens.*





ACTION OF POLYANIONE POLYSACCHARIDES FROM THE BROWN SEAWEEDS *FUCUS EVANESCENS* AND THEIR MODIFIED ANALOGS ON REPRODUCTIVE FUNCTION AND DEVELOPMENT SEA URCHIN EMBRYOS *STRONGULOCENTROTUS INTERMEDIUS*

Kiseleva, M.I., Bakunina, I. Y., Kusaykin, M.I., Zvyagintseva, T.N.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail:MIKiseleva@mail.ru

Fucoidans are sulfated heteropolysaccharides from brown seaweeds. They have a wide spectrum of biological action: antibacterial, anticoagulant, antithrombotic, antiinflammatory, antitumor, contraceptive, and antiviral properties. Brown algae coexisting with sea urchin in the same biocenosis represent one of the basic sources of its feed. Earlier the effect of brown algae polysaccharides, such as fucoidans, 1,3;1,6-β-D-glucans and uronan, on the developing of sea urchin embryos *Strongilocentrotus intermedius,* has been studied [1,2,3].

In the present study the effect of brown algae fucoidans on the development and survival of the sea urchin embryos, *S. intermedius*, has been evaluated.

Sea urchins *S. intermedius* were collected from the Peter the Great Bay, Sea of Japan, Khasansky region, Prymorsky kraii, Russia. Brown seaweeds *Fucus evanescens* were collected in Iturup Island, Sea of Okhotsk.

Native fucoidans representing a polysaccharide\polyphenol complexes, differing in their monosaccharide composition, partly desulfated fucoidans, connected to polyphenols; high sulfated fucoidans, and polysaccharides partly desulfated, liberated out of polyphenols, as well as mannuronan were investigated by us.

As was shown, both native and modified polyanione polysaccharides from *F. evanescens* inhibited process of a sea urchin fertilisation. The partly desulfated fucoidans purified from polyphenols showed the most inhibition action on fertilization. Higher sulfated, strongly connected to polyphenols fucoidans, as wall as mannuronan shown weak inhibition action on process of fertilisation.

Effect of fucoidans on developing see urchin embryos was studied in two variants. The first, adding of the fucoidans in incubatory mixture in time of fertilisation (zygote stage) or the second, hatched blastula embryos (stage of late blastula) were treated with polysaccharides of different concentrations. Fucoidans, strongly connected to polyphenols, appreciably increased survivability of embryos. Concentration of polysacchrides increasing survivability of embryos was lower when cells were treated during fertilization. Fucoidan mainly consisting of fucose residues acted on survival of embryos at low concentrations. However, increase of the polysaccharide concentration resulted in lagging of development and incipience of anomalies of embryos. It is possible to note, that practically all polysaccharides studied excepting native and desulfated fucoidans, strongly connected with polyphenols caused anomalies appearing at early stages of development after addition of substances during fertilisation. Partly desulfated fucoidans, purified from polyphenols were the most toxic substance in these experiments, as well as at experiments on influence of fucoidans on fertilisation.

Thus, the strongest inhibitor, especialy concerning process of fertilisation, is partly desulfated fucoidan liberated out from polyphenols. The most active fucoidans rendering positive action on viability of developing embryos are high sulfated, connected to polyphenols fucoidans.

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UV-PROTECTIVE PROPERTIES OF GLUCAN FROM MUSSELS

[1]Kipryushina,Yu.O., [2]Odintsova, N.A., [3]Wei Li, [4]Molchanova, V.I., [4]Lukyanov, P.A.

[1]Far -Eastern State University, [2]Institute of Marine Biology, [3] Dalyan Fishery University (China), [4] Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: paluk@mail.ru

An appreciable destruction of an ozone cloud convinced with emission of industrial pollution in an atmosphere that has led to increase in intensity of ultra-violet radiation of diapason C (UV-C) was detected during last 25 years. As known UV-C radiation destroy DNA and proteins, also it leads to formation of free radicals.

The polysaccharide with molecular mass 2 MDa named Mytilan was isolated from a mantle of trade kinds of mussels (*Crenomytilus grayanus, Mytilus edulis, M. trossulus)*, which is a glycogen-like 1,4; 1,6-*a*-D-glucan with high degree of branching of a carbohydrate chain and presence of a small number of 1,2 and 1,3-a-D-glucose residues in branching points.

This substance was found in medicobiologic researches to be nontoxic (LD_{50} more 1 g/kg), don't show irritating or allergic action at the contact with skin, possess a marked antiphlogistic effect and have possibility to reinforce the immunity to bacterial and virus infection also. In addition, Mytilan was shown to have anti-tumorous activity. In this work we investigated UV-protective effect of Mytilan for marine invertebrate larvae at early development stages. It is known that early developmental stages of hydrobiontes are sensible extremely to the influence of disturbing factors. These factors can result in different developmental disturbances, which may be identify easy and estimate quantitatively. This polysaccharide was found to possess intense protective effect at preincubation of larvae at during 5-10 hours until uviolizing. The elevated effectiveness of this medication was observed at the using of extremely small doses (2-200 mkg/ml). After 3-4h- uviolizing (at 10-15°C) a number of viable larvae was demonstrated to be in 2-4 times more at the presence of Mytilan. Moreover, the developmental disturbances, pronounced in control UV-radiation exposed larvae, were minimal in those variants, in which Mytilan was added in sea water. In one day after uviolizing a number of viable larvae at the presence of mytilan was in 1.5-2.5 times more than in sea water.

It is revealed, that this polysaccharide possesses protective effect for peripheral white blood cells treated 0.5-3 hours before UV irradiation in sublethal doze also. Efficiency of Mytilan is shown in low concentration up to 200 mkg/ml, thus the cells survival increases on 20-40% in comparison with control. The incubation of cells with Mytilan in sublethal doze of UV-C irradiation reduces NO-synthase activity and reactive oxygen species generation. It was found that an intracellular esterase activity in the lymphocytes culture increases at incubation with Mytilan before UV-C irradiation in comparison with the control cells.

As was shown by us Mytilan can protect cells from the damages caused UV irradiation.

The work was supported in part by grant "НШ-2383.2008.4"

OSTEOPROTEGERIN AND OSTEOPENIC SYNDROME IN CHRONIC OBSTRUCTIVE LUNG DISEASE

[1,2]Kochetkova, E. [3]Maistrovskai, Y. [3]Nevzorova, V . [3]Albavichus, S. [1]Kessler, R. [1]Massard, G. [3]Ugai, L. [3]Pestrikova, T.

[1]Hopitaux Universitaires de Strasbourg, France

[2]Filial of Institute of Genetice of Tomsk's Science Center SB RAMS, Vladivostok

[3]Vladivostok State Medical University, Russia

Hopitaux Universitaires de Strasbourg

67091 Strasbourg

e-mail: zkochetkova@mail.ru

Osteoprotegerin (OPG) is a glycoprotein that belongs to the TNF-receptor superfamily. It is a part of a newly described cytokine system that is important in the control of osteoclast maturation, where a RANK-Ligand (receptor activator of nuclear factor kappaB-ligand) on osteoblasts binds to RANK on osteoclast precursors, leading to their differentiation. In vitro and animal studies have revealed the role of OPG as a decoy receptor that neutralizes the effect of RANKL on the differentiation and activation of osteoclasts. Howere, the role of the OPG- RANKL system in pulmonary osteoporosis is controversial.

Aim: to investigate the possible relationship between circulating levels of OPG, bone turnover markers, bone mineral density (BMD) in chronic obstructive lung disease (COPD).

Material and methods: 45 patients with COPD aged 44 to 58 years were examined. BMD was measured in all patients by means of dual-energy X-ray absorptiometry (DXA) at the lumbar spine (LS), left femur (LF) using a Lunar Prodigy Densitometer (USA). Patients' individual BMD values were axpressed as T-scores and compared with these reference data. Osteopenia was defined according to the WHO guidelines as T-score <-1, osteoporosis, <-2.5 SD. Conrol group consisted of 35 health individuals of same age. We estimated TNF-alpha, OPG and CrossLaps (marker of bone resorbtion) in the blood.

Results: Osteopenic syndrome (T-score <-1SD) was observed in 34/45 (76%) patients with COPD. Of them 13/45 (29%) had osteoporosis both at LS and LF. Serum levels of TNF-alpha were increased in COPD in comparison with control group ($p<0.01$) and it correlated positively with CrossLaps ($r=0.52$; $p=0.042$) but negatively with OPG ($r=0.44$; $p=0.032$). OPG was found to be independently associated with osteoporosis.

Conclusion: In summary, we found low bone density, high concentrations of TNF-alpha, low concentrations of OPG, and high rates of bone turnover markers (CrossLaps) in patients with COPD. We hypothesize a pathogenetic role of TNF-alpha and OPG in the process underlying the bone loss in this group of patients.

FERRUGINOUS PROTEINS FROM MARINE HYDROBIONTS AS A NEW COMPONENT FOR BIOLOGICALLY ACTIVE SUPPLEMENTS

Kofanova, N.N., Rutskova, T.A., Artyukov, A.A., Coopera, E.V., Kozlovskaya, E.P.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: elna@piboc.dvo.ru

The provision of tissues with oxygen is fundamental and vital condition of existence of any organism. Hemoglobin carries out this function in the vertebrates, hemoglobin, hemocyanin, chlorocruorin, hemoerythrin, – in the invertebrates.

At present time blood of cattle is a basic source of ferruginous proteins for the production of drugs and biologically active food supplements. Last years search for alternative sources of such proteins, phylogenetically removed from the mammalians and not amenable to virus diseases dangerous for human are conducted. A trade mollusk *Scapharca broughtoni* could be such a source containing large quantities of hemoproteides. We developed a method for isolation of ferruginous proteins from wastes after industrial processing of this mollusk hemolymph and studied its physico-chemical properties.

The spectrum of the protein purified has absorption peaks at 277, 355, 412, and 531 nm that corresponds to peaks of oxihemoglobin and are characteristic for chromoproteids, containing a ferric porphyrin group. According to the atomic absorption spectral analysis, the preparation contains 2.09 mg ferrum in 1 g of dry substance that is comparable with the contents of ferrum in the organs participating in breath, gas exchange and oxidizing clearing. Molecular weight of native oxihemoglobulin from *Scapharca broughtoni* is more than 300000 Da, and a monomer wol.wgt. is 15000 Da.

Taking into account the high contents of iron in this protein, availability of raw material, and simplicity of its isolation, we developed a method of production of biologically active food supplements containing 3 – 5 % of iron.

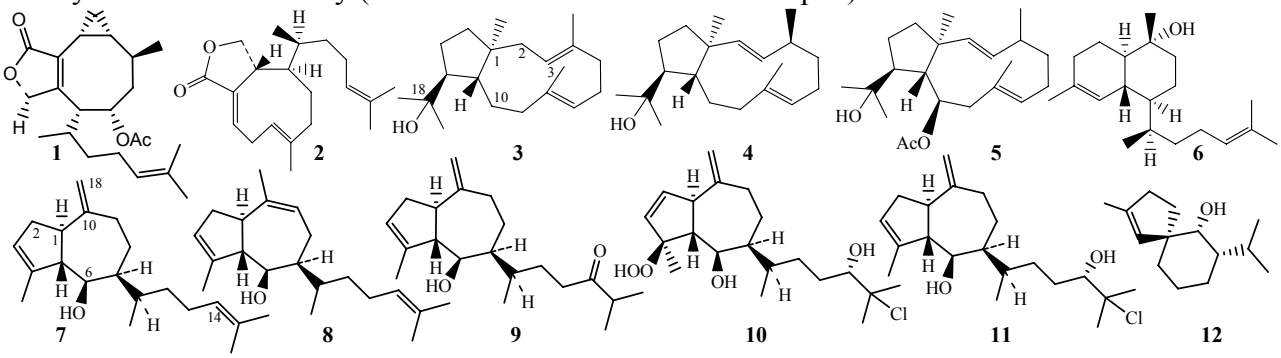
BIOACTIVE DITERPENES FROM THE BROWN ALGA *DICTYOTA DICHOTOMA*

Kolesnikova, S.A., Dyshlovoy, S.A., Lyakhova, E.G., Fedorov, S.N.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia

e-mail: sovin81@inbox.ru

Brown algae *Dictyota dichotoma* is known to be a prolific source of different terpenoid secondary metabolites possessing antibiotic, antifungal, antiviral, cytotoxic and antitumor activities. Herein we report the results of the cell viability assay carried out with eleven diterpenes (**1-11**) and one sesquiterpenoid alcohol (**12**) isolated from two samples of *D. dichotoma*, collected in August 2004 and July 2006 in Troitsa Bay (the Russian shore of the Sea of Japan).



All diterpenes belonging to three structural groups: xenicanes (**1**, **2**), dolabellanes (**3-5**) and so called "extended sesquiterpenes" (**6-11**) as well as sesquiterpene axenol (**12**) showed moderate activity against human tumor cell line (HeLa) and normal mouse epithelial cells (JB6 P^+ Cl41) (Table).

Xenicanes **1** and **2** were more active against normal mouse epithelial cells than against human tumor cells. It's obviously that double decrease of cytotoxicity of dolabellane **4** against HeLa cells in comparison with that of dolabellane **3** can be explained by the shift of double bond from position 2 to position 3. On the other hand, the additional acetoxy group in position 10 of compound **5** significantly increased cytotoxicity.

The study of bioactivity of hydroazulenes **7-11** showed that the presence of the exo-methylen group in position 10 (compound **7**) increases cytotoxicity in comparison with substances with 9-endo-double bond (compound **8**). The substitution of 14-double bond in the side chain of compound **7** by keto-group (compound **9**) didn't change the cytotoxicity significantly. At the same time the reduction of keto-group (compound **9**) to hydroxyl group (compound **11**) strongly decreased the cytotoxic activity in spite of the presence of potentially bioactive chlorine substituent. The presence of hydroperoxide group in compound **10** so significantly increased the cytotoxicity, that **10** is the most active among hydroazulenic compounds **7-11**.

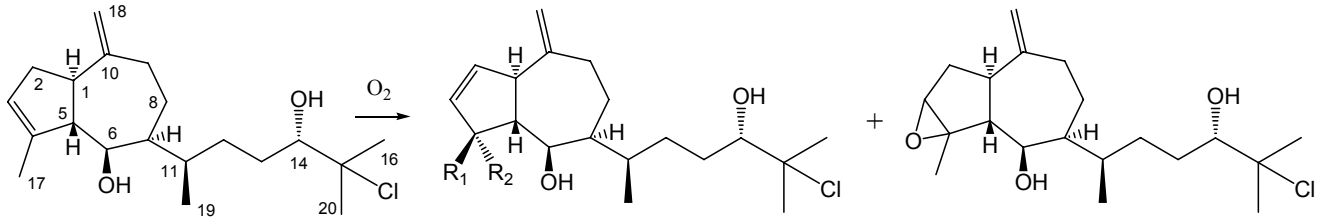
Table. The cytotoxicity of compounds **1-12** against human tumor (HeLa) and mouse epithelial (JB6 Cl 41) cell lines measured using the MTS method.

Compound	IC_{50}, μM	
	HeLa	JB6 P^+ Cl41
Acetoxycrenulide (**1**)	388	157
Dictyolactone (**2**)	497	321
18-Hydroxy-3,7-dolabelladiene (**3**)	74	85
18-Hydroxy-2,7-dolabelladiene (**4**)	146	124
10-Acetoxy-18-Hydroxy-2,7-dolabelladiene (**5**)	59	94
Dictyotin B (**6**)	73	70
Pachidictyol A (**7**)	98	110
Isopachidictyol A (**8**)	162	129
Dictyone (**9**)	108	115
Compound **10**	71	68
Dictyol J (**11**)	246	147
Axenol (**12**)	182	237

HALOGENATED DITERPENES FROM THE BROWN ALGA *DICTYOTA DICHOTOMA*

Kolesnikova, S.A., Lyakhova, E.G., Kalinovsky, A.I., Dmitrenok, P.A., Dyshlovoy, S.A., Stonik, V.A.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: sovin81@inbox.ru

The brown algae belonging to the genus *Dictyota* are known to be prolific sources of different secondary metabolites. Taking into account that the same species of this genus from different localities may contain different sets of compounds, we decided to study the brown alga *D. dichotoma*, growing in Troitsa Bay of the Peter the Great Bay. Our study on the population of *D. dichotoma* collected in July 2006 using different cromatographical procedures followed by NMR spectroscopy and mass-spectrometry gave a number of previously known compounds including pentadecane, (+)-1,5-cyclo-5,8,9,10-tetrahydroerogorgiaene, isopachydictyol A, axenol, 18-hydroxy-3,7-dolabelladiene, 18-hydroxy-2,7-dolabelladiene, 10-acetoxy-18-hydroxy-2,7-dolabelladiene, dictyone, acetoxycrenulide, ent-erogorgiaene, squalene, fucosterol, saringosterol and dictyol J (**1**). In addition, a new natural halogenated diterpenoidal hydroperoxide (**2**), whose structures and relative stereochemistry have been determined by 1D and 2D NMR spectroscopy (^{1}H-^{1}H COSY, HMBC, HSQC, NOESY), HRMS and chemical transformation was isolated.



1

2 R_1 = OOH, R_2 = CH_3

3 R_1 = CH_3, R_2 = OOH

4

The molecular formula of compound (**2**) was determined as $C_{20}H_{33}O_4Cl$ by high-resolution mass spectrometry and NMR. The NMR spectra of **2** were partly identical to those of previously known dictyol J (**1**). However the ^{13}C NMR spectrum indicated the presence of disubstituted double bond and four carbons bonded to heteroatoms. Fragmentation ion at m/z 159 characteristic for hydroazulenoid diterpenes was not present in the mass spectrum of **2**, suggesting that structural differences resided within the bicyclic moiety. The detailed analysis of NMR (^{1}H-^{1}H COSY, DEPT, NOESY, HMBC, HMQC) and HRMS spectra allowed to establish the structure **2** for isolated compound.

It was suggested that compound **2** is an oxygenated product of dictyol J (**1**). To examine this proposition we carried out the oxygenation of natural compound **1** with air. As result, we have isolated the mixture (2:1) of compounds **2** and **3** as well as product **4** whose structures were determined by NMR and mass-spectrometry. The results of this experiment showed natural compound **2** to have the same relative stereochemistry of all asymmetric centres besides C-4 as those of dictyol J (**1**). The relative configuration of C-4 was established by NOESY.

The cytotoxicity of dictyol J (**1**) and compound **2** for several human tumor and normal mouse epithelial cell lines JB6 Cl 41 was tested using the MTS method. Both compounds showed moderate activity against cell lines HL-60 and were significantly less cytotoxic against MDA-MB-231. Dictyol J (**1**) was two times more citoxic against HeLa than against normal mouse epithelial cell lines JB6 Cl 41.

Thus we have found two halogenated diterpenoids, one of them was a new. The new compound **2** was a first representative containing rare hydroperoxide group. It is a second time occurrence of halogenated natural products from brown alga of the family Dictiotaceae.

MOLECULAR STRUCTURE OF CARBOHYDRATE O-ANTIGENS FROM SOME GRAM-NEGATIVE MARINE BACTERIA

Komandrova, N.A., Tomshich, S.V., Nazarenko, E.L., Gorshkova R.P.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: komand@piboc.dvo.ru

Gram-negative bacteria are ubiqitions in marine environments. As in the case of other microorganisms from sea habitats, they represent an interesting field of research, being a valuable source of natural substances provided with powerful bioactivity. Among the compounds that marine bacteria are able to synthesize, we can mention a wide range of antibiotics, toxins and antitoxins, antitumor and antimicrobial agents and enzymes with a wide spectrum of action.

The majority of the Gram-negative marine bacteria investigated to date belong to the *Alteromonadaceae* family of the γ-subclass of the proteobacteria that encompasses, among others, the genera *Shewanella, Alteromonas, Pseudoalteromonas, Glaceocola* and *Idiomarina.* More recently, studies have started, focusing on species from the *Cytophaga-Flavobacterium-Bacteroides* phylum.

Gram-negative bacteria are essential components of marine environments and can be encountered in diverse habitats, including coastal and open water areas, deep-sea and hydrothermal vents, bottom sediments as well as marine plants and animals, with which they can establish symbiotic or pathogenic interactions.

In nearly all Gram-negative and in all marine Gram-negative bacteria, the outermost layer of the cell envelope is constituted by the outer membrane, an asymmetric bilayer in whose outer leaflet are embedded Lipopolysaccharides (LPS). These characteristic and vital molecules represent the contact between the bacterial cell and the surrounding environments, therefore it is plausible that many of the functional changes induced by the harsh habitats can target LPS structure.

Investigations were shown that marine Gram-negative bacteria of different genera produce of superficial antigenic carbohydrate biopolymers having unique structures. These bioglycans contain of unusual acidic monosaccharides, 6-deoxyamino- and keto- sugars, higher sugars or acidic non-carbohydrate substituents, i.e. malic acid in the O-polysaccharide from *Shewanella algae BrY* or the peculiar monosaccharide *Shewanellose*, a novel C-branched sugar [2-acetamido-2,6-dideoxy-4-C-(3'carboxamide-2',2'-dihydroxypropyl)-D-galactose, She] first found in the O-polysaccharide from *S. patrefaciens* A6, together with a derivative of the 8-epilegionaminic acid.

The core oligosaccharides from the two new species from *Pseudoalteromonas, P. issachenkonii* KMM 3549^T, *P. carrageenovora* IAM 12662^T have been recently established. They are composed by a mixture of three glycoforms, differing for the length of the sugar chain and the phosphorylation pattern, and are characterized by a strong accumulation of phosphate groups in the lipid A-core portion. From the structural investigation on the core oligosaccharides of LPSs from *Shewanella,* interesting information have emerged: Kdo residue is replaced by an 8-amino derivative (Kdo8N0, as junction of the OS with lipid A. This carbohydrate residue may be considered as a taxonomic marker for the genus.

The newly defined *Alteromonas* genus comprises few validly described species: *A. macleodii, A. marina, A. stellipolaris, A. litorea* and *A. addita.* The structure investigation of the LPS structures from bacteria belonging to this genus has only recenyly begun, and, up to now, only two structures have been given, from *A. macleodii* ATCC 27126^T and from *A. addita* KMM 3600^T Interestingly, in both cases the bacteria have been found to produce only a R-LPS, provided with an extremely short oligosaccharide chain with a high negative charge density.

This chemical structural information of carbohydrate-containing biopolymers may be useful in classification of Gram-negative marine bacteria and elaborating the current concepts regarding the organization and mechanisms of functioning of threir cell wall.

THE CORECTION OF LIPID AND CARBOHYDRATE METABOLIC USING BIOANTIOXIDANTS AND POLAR LIPIDS FROM SEA HYDROBIONTES

Krivoshapko O.N.[1], Popov A.M.[1], Artyukov A.A.[1], Kostetsky E.Y.[2]
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
[2]Far-Eastern National University, Vladivostok, 690000 Oktyabrskaya, 27, Russia
e-mail: popovam@piboc.dvo.ru

In present work was realized isolation of total mixture phospho- and glycolipids from sea macrophytes of *Sargassum pallidum*, *Ulva fenestrata*, *Zostera marina* and their fatty acid composition was determined. The medical-preventive activity specified above mixtures polar lipids and natural antioxidants echinochrome A from flat sea urchin *Scaphechinus mirabilis* and polyphenolic complex from sea grass *Zostera marina* at carbohydrate and lipid metabolic imbalances were studied. On experimental model atherosclerosis and diabetes were revealed optimum compositions of the mixtures polar lipids and antioxidants, which possess high medical-preventive activity. The supposed mechanisms of the action polar lipids, containing different polyunsaturated fatty acids, and antioxidants studied are presented. On the base these compositions is expected creation new supplements and medicines.

EFFECTS OF METALLIC MERCURY VAPOUR ON LIPID METABOLISM AND ANTIOXIDANT SYSTEM INDICES

Kudaeva, I., Masnavieva, L., Budarina, L.

Institute of Occupational Health and Human Ecology, Scientific Center of Medical Ecology, East-Siberian Scientific Center, Siberian Division of the Russian Academy of Medical Sciences, Russia, Angarsk,665827/ host box 1170

e-mail: kudaeva_irina@mail.ru, imt@irmail.ru

The toxicant exposure leads to the alteration in developing the biochemical processes in organism inducing the pathology occurrence. The disturbances in lipid metabolism developed at the background of the decrease in the antioxidant protection which are followed by the early disease development of the cardio-vascular system in the exposure to a complex of toxic substances. (Kudaeva I.V. et al., 2002; Budarina L.A. et al., 2007). The arterial hypertension is known to be one of the more wide-spread pathologies which are registered before forming the expressed neurological disorders in the persons exposed to metallic mercury. The development of pro-atherogenic disturbances is found to be one of the key links in the pathogenesis of the latter. Taking into account this, the aim of this study was to investigate the indices of lipid metabolism, the antioxidant system and nitrogen oxide in the workers exposed to metallic mercury vapour. To realize this aim, 59 workers with the work duration under harmful conditions of exposure to mercury for 15 and more years (Group II-), 9 persons with a newly revealed diagnosis of chronic mercury intoxication (CMI) – (Group III), 36 patients with a long-term effect of CMI – (Group IV) have been examined. Group I (control group) consisted of 45 persons matched on the age and the work duration who had no occupational exposure to chemical substances. The indices of lipid metabolism have been studied using the unificated methods, the nitrogen oxide level – based on a summery quantity off its stabile metabolites, the reduced glutathione content in the whole blood – the colorimetric method, the super oxide dismutase activity – based on the degree of delaying the adrenaline auto oxidation in the alkaline buffer. The statistical processing of the findings was carried out using the Kit of applied programs “STATISTICA”. The studies performed allowed to reveal that the alteration dynamic in the lipid metabolism indices in all the persons exposed to mercury vapour or without this exposure was found to be the one-directed one.

So, the increase in common cholesterin in the representatives of the given groups, on the average 14% compared with the control values (P=0.016), occurred due to its fraction in lipoproteides of a very low density (P= 0.02) at the background of decrease cholesterin in the lipoproteide of high density (P=0.006). Thereby, the increase in the triglyceride concentration 1.5 times was observed in the persons of Cohorts II and IV and more than 2 times in the patients with a newly revealed diagnosis of CMI compared with the control values (P=0.002). As for the disorders in lipid-transport system, it should be noted that there was the decrease in the relative number of lipoproteides of high density in the persons examined of Group II-IV by 26-33% from the control level (P=0.0002) which was more expressed in the patients with CMI a long-term period after exposure. The disturbances observed were followed by the increase in the content of the lipoproteide fraction of a very low density more than 2 times compared with the data of the control Group (P=0.0003). The degree of the pronouncement of the observed changes prevailed in the workers with a newly revealed diagnosis of an occupational disease. It should be noted that the nitrogen oxide level fluctuations compared with the control values (P=0.037) were displayed in its decrease in the workers of Group II and in the increase in the representatives of two other groups. Thereto, superoxide dismutase activity decreased significantly (P=0.0000) in all the persons exposed to the toxicant (25.6%) or having the diagnosis of CMI (23.6-31.5%).

These disorders developed at the background of the decrease in the reduced glutathione concentration in the whole blood (P=0.0001) of the persons of Groups II and III 18.6% and 25.5%, respectively. The factor of stopping the exposure to the toxicant was found to promote the content recovery of this antioxidant.

Thus, the long-term exposure to the metallic mercury vapour leads to the pro-atherogenic disturbances of lipid metabolism, the alteration in the nitrogen oxide level and the decrease in the antioxidant protection.

MARINE MICROORGANISM METABOLITES AS BIOLOGICAL TOOLS AND BIOMEDICAL SOURCES

Kuznetsova, T.A

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: kuztata@piboc.dvo.ru

Marine microorganisms have developed unique metabolic and physiological capabilities that ensure survival in extreme habitat which is characterized by high salinity and low concentrations of organic matter, low temperature and high hydrostatic pressure as well as specific marine nutrients. The existence of marine microorganisms was first reported in the late of the 19-th century, and their microbial growth and the products of their metabolic activities differ considerably from those of terrestrial microorganisms. We now know that some marine microorganisms produce structurally-novel, secondary bioactive metabolites. The discovery of novel drugs from the marine microorganisms is the fund for development of marine biotechnology, which may open new opportunities for using and management of the biological resources from the ocean. As part of our interest in exploring the biologically active secondary metabolites of marine microorganisms obtained from the marine environment we have investigated several *Pseudoalteromonas*, *Bacillus*, *Vibrio* and *Streptomyces* strains and found antibacterial, cytotoxic compounds, biosurfactants and in their extracts. The chemical structures of cyclic peptides and depsipeptides, gentiobiosyl diglycerides, isocumarins, brominated diphenyl ethers were elucidated by high-resolution mass spectrometry and 2D NMR spectroscopy. The compound with important ecological implication were discovered. We have tested also about one thousand strains of marine fungi isolated from the marine invertebrates, bottom sediments and from alga. The sources of antimicrobial compounds against Gram positive and Gram negative bacteria. The chemical structures of the diterpene glycosides from *Acremonium striatisporum,* terpenes from *Humicola fuscoatra* KMM 4629, indole alkaloids fron marine fungus isolate of *Penicillium janthinellum* Biourge were determined on the basis of the mass and NMR data. The compounds with the potencial antitumor activity were isolated with the highest yield from the marine isolate of the fungus *Aspergillus fumigatus* KMM 4631.

So, marine microorganisms have proven to be a new promising source for bioactive substances. Research to date indicates that further studies on marine microorganisms may be confidently expected to yield new bioactive compounds that are applicable to drug development or useful for basic research in life sciences.

METABOLITES – THE CHEMICAL LANGUAGE OF MICROBE

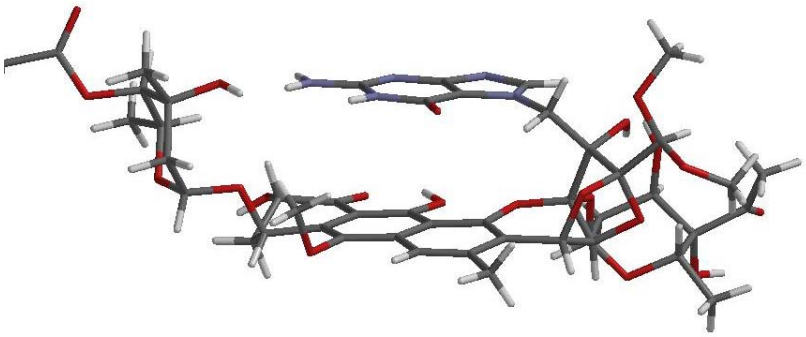
Hartmut LAATSCH

Department of Organic and Biomolecular Cemistry, University of Göttingen
Tammannstrasse 2, D-37077 Göttingen, Germany; hlaatsc@gwdg.de

Natural products are words and messages in a global communication system of species interactions. They have a special meaning and will effect an answer, which is not urgently constant, but depends on the given situation. We just begin to understand this language of nature and try to compile the vocabulary and to decipher the grammar. Natural products are weapons and defence systems, attractants or repellents, or just communication signals, which are important for the survival of species. Also resistance development of bacteria against antibiotics is such a logical and unavoidable reply on environmental effects, which we can only overcome by a better understanding of the 'microbial conversation'.

In consequence, the investigation of ecological interactions and a continuous and efficient search for new natural products with potential application in medicine is a steady and indispensable task. However, since the number of natural products is limited, every newly isolated product will diminish the chances to find further hits. And every re-isolated known compound is an avoidable loss of time and money. Two techniques are widely applied to overcome these problems. The first one is the activity-directed high throughput screening, which is an expensive and fully automated, mainly industrial process; the other one is the so-called chemical screening.

We are using a third technique, a type of dragnet search, where we compare easily accessible data of isolated and purified compounds with comprehensive databases of more than 50.000 marine and microbial natural products, AntiBase and MarinLit (= AntiMarin). Comparison of sub-structures, NMR, MS, and UV data allows a very fast decision as to whether a given natural product is already known or not, which enables us to save time and resources and to avoid frustration, and to concentrate on the really new topics.



X-ray Structure of Gutingimycin

This presentation will explain modern dereplication techniques using MS/MS, 2D NMR and database methods. The procedure will be highlighted by some simple and some very complex examples from our ongoing research on marine bacteria.

A COMPARATIVE STUDY OF THE BIOACTIVE METABOLITES OF MARINE INVERTEBRATES FROM MICRONESIAN AND KOREAN WATERS.

Hyi-Seung Lee
Marine Natural Products Laboratory, Korea Ocean Research & Development Institute, Ansan PO Box 29, Seoul 425-600, Korea
e-mail: hslee@kordi.re.kr

Marine natural products with their obvious bioactivities and unique structural features have attracted the attention of biologists and chemists the world over for the past several decades. Especially, marine invertebrates such as sponges, tunicates and mollusks have provided the largest number of marine-derived secondary constituents including some of the most interesting drug candidates.

During the course of our search for bioactive secondary metabolites, we collected 74 species of marine invertebrates from Micronesian and Korean waters and tested for antimicrobial, antiviral and anticancer activities from their extracts. The present study investigated aspects of the biologically active natural products of marine invertebrates, which are collected from two different sea areas. And studies on the chemical investigation of some invertebrates led to the isolation of alkaloids, acetylenes, phenols and terpenes. The structures of the novel metabolites have been elucidated by combined chemical and spectral methods.

APPLICATION OF MODELING AND MOLECULAR DOCKING FOR STUDY OF ENZYME 3D-STRUCTURES

[1]Likhatskaya, G.N, [2]Trifonov, E.V., [1]Kovalchuk, S.N., [1]Balabanova, L.A., [1]Bakunina, I.Y., [1]Petruk, S.V., [3]Turashev, A.D., [1]Zvyagintseva, T.N., [2]Nurminsky, E.A., [3]Maksimenko, A.V., [1]Rasskazov, V.A.

[1]Pacific Institute of Bioorganic Chemistry of Far Eastern Branch of Russian Academy of Sciences, 690022, Vladivostok, Prospect 100 let Vladivostoku 159, fax: (4232)31405, e-mail:galinlik@piboc.dvo.ru

[2]-Institute for Automation and Control Processes,690041, Vladivostok, Radio street, 5; e-mail:trif@dvo.ru

[3] Institute of Experimental Cardiology, Russian Cardiology Research-and-Production, 121552, Moscow, 3rd Cherepkovskaya street, 15 A; fax: (495)4146699; e-mail:cclibr@comcor.ru

The objects of our study were the following enzymes: laminarinases, O-glycoside hydrolases that cleave water soluble polysaccharides laminarans, from the crystalline style of commercial molluscs *Pseudocardium sachalinensis* (LIV) and *Chlamys albidus* (Lo); Ca^{2+}-, Mg^{2+}-dependent enzyme depolymerising nucleic acids with high thermal stability and specificity to the double stranded DNA (DSN) from the king crab *Paralithodes camtchatica*; bovine testicular hyaluronidase(BTH); alkaline phosphatase (AP) from marine bacterium *Cobetia marina* strain KMM 296 and alpha-galactosidase (AG) from *Pseudoalteromonas* sp. KMM 701. The primary structures of these enzymes have been recently determined by the molecular cloning methods (GenBank accession numbers AY308829, DQ093347, AF520591, AY297029, DQ435608 and DQ530422), but up to date experimental data about their 3D-structures have not been obtained. Fold recognitions of marine enzymes were carried out using 3D-PSSM, FUGUE and PHYRE servers. It was found that enzymes have folds with 100% confidence to enzymes having known crystal structures. Homology models of enzymes were generated by SWISS-MODEL server and homology model module of program MOE. Energy minimizations of the models were carried out using program GROMACS 3.3.1. The putative N-glycosilation sites in enzymes were predicted by NetNGlyc 1.0 server. Models of laminarinases Lo and LIV were generated on the base the crystal structure of endo-1,3(4)-beta-glucanase laminarinase (Lam16A) from *Phanerochaete chrysosporium* (code PDB 2CL2). Models of DSN, AP and AG were generated on the base of crystal structures of endonuclease from *Serratia marcescens* (code PDB 1G8T), alkaline phosphatase from northern shrimp *Pandalus borealis* (code PDB 1K7H) and alpha-galactosidase from *Thermotoga maritima* (code PDB 1ZY9) accordingly. Model of native BTH was generated on the base of crystal structure human hyaluronidase-1 (code PDB 2PE4). Model of BTH modified with chondroitin sulfate was obtained using program MOE. Enzyme models were used for prediction of the structures of enzyme-substrate and enzyme-inhibitor complexes by molecular docking approach. Models of complexes were building with programs GRAMM 1.03, Docking module of MOE 2007.09 and Autodock4. Atomistic details of marine enzymes active sites, substrate binding subsites, inhibitors and metal binding sites were obtained. Enzymes 3D-structural data will allow an understanding better of the structure-function relationships and regulation of enzyme activities. The interactions of BTH and chondroitin sulfate-modified BTH with glycosaminoglycan ligands, mono- and disaccharides were studied in silico for better understanding glycation of native and modified hyaluronidase. Key residues (Lys77, Arg152, Lys190, Pro301) for BTH glycation by mono- and disaccharides, which are important for understanding molecular regulatory mechanisms of hyaluronidase function and development of stabilized recombinant forms of the enzyme for medical use were found.

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IDENTIFICATION AND QUALITY CONTROL OF HEALTHCARE PRODUCTS OBTAINED FROM VIETNAMESE SEED OILS

Pham Quoc Long[1*], Matthaus B. [2], Imbs A.B.[3]

1. Institute of Natural Products Chemistry, VAST, Hanoi, Vietnam
18-Hoang Quoc Viet, Hanoi, Vietnam; e-mail: mar.biochem@fpt.vn
2. Institute for Lipid Research BAGKF, Muenster, Germany
3. A.V Zhirmunsky Institute of Marine Biology FER RAS, Vladivostok, Russia

Vietnam, China, and Korea successfully integrated a lot of products of traditional medicine into their healthcare systems. About 70 countries the world over have national regulations for herbal medicines, but different approaches to licensing, dispensing, manufacturing, and trading these products are applied in different countries. The approaches to the identification and quality control of healthcare products should be developed and unified.

The great variety of fruits and vegetable is known in Vietnam. The compositions of tocopherols, phenolic compounds, sterols, fatty acids (FAs), triglycerols, and other lipids from seed oil of these renewable resources require in-depth investigations. Lipids are of outstanding importance for human nutrition being the source of energy, fat-soluble vitamins, and essential FAs. In Vietnam, seed lipids are widely used both as traditional medicines and as food. Therefore, it is necessary to develop reliable methods for the identification and standardization of sources of oils, FAs, and other seed lipids, which are considered as natural and synthesized healthcare products in Vietnam.

Herbal medicines are an extraordinary complex chemical composition, and rapid identification of all its active compounds is still unsolved problem. At present *Fingerprint Chromatographic Analysis,* which combines data of TLC, GC-MS, LC-MS, HLPC, LC-NMR, etc., is the best available approach to the identification and qualification of medicinal herbs.

Lipids from plant seeds may contain unusual highly specific FAs, which are often correlated with plant families. Other FAs may be characteristic for certain sub-families or species of Vietnamese plants. Modern capillary gas chromatography (GC) enables high-resolution "fingerprint" separations of FAs as well as the identification of "unusual" FAs. Such "fingerprints" can be applied to check unusual FAs in *Chemotaxonomic significance and quality control* that may indicate the presence of plant seed oil in medicines and their type. In this case, FAs are chemotaxonomic markers of plants.

In the nearest future, *Fingerprint Chromatograms* will become a standard technique for the qualitative evaluation of the herbal medicines and other products, as well as for the analysis of raw materials.

APPLICATION OF NUCLEAR MAGNETIC RESONANCE TECHNIQUES TO STUDY A BIOCATALYTIC REACTOR AND HETEROGENEOUS BIOCATALYTIC PROCESSES

Lysova, A.A.[1,2,3], Koptyug, I.I.[1,3], Koptyug, I.V.[1]
[1]*International Tomography Center SB RAS, Novosibisrk, str. Institutskaya 3A, 630090, Russia;*
[2]*Boreskov Institute of Catalysis SB RAS, Novosibisk, pr. Ak. Lavrentieva 5, 630090, Russia;*
[3]*Novosibirsk State University, Novosibirsk, str. Pirogova 2, 630090, Russia;*
fax: +7 383 3331399; e-mail: lysova@tomo.nsc.ru

Many industrial chemical processes are carried out by microorganisms or biochemically active substances produced by these microorganisms (biocatalysts). Biocatalysts can be dissolved in a homogeneous reaction mixture and, therefore, participate in a homogeneous biocatalytic process, or they can be immobilized on a porous support and carry out a heterogeneous biocatalytic process. The realization of a biocatalytic process in a heterogeneous mode has an advantage over a homogeneous mode because an immobilized biocatalyst can be easily removed from the reaction mixture after completion of the process. However, the use of a heterogeneous biocatalyst brings also some problems typical for a common heterogeneous catalysis, such as the non-uniform distribution of an active component (cells, enzymes, etc.) in the pores of a heterogeneous support, the non-uniform distribution of a reagent within a biocatalyst bed, the non-uniformity of mass transfer coefficients, velocity field, etc., in a heterogeneous biocatalytic reactor, resulting in a imperfect utilization of a biocatalyst.

One of the techniques that can provide us with the detailed information about the non-uniformity of a heterogeneous biocatalytic process taking place in a functioning biocatalytic reactor is nuclear magnetic resonance (NMR) imaging. NMR imaging allows one to get the information about the distribution of a liquid phase, diffusion and dispersion coefficients, flow velocities, temperatures, etc., in the object of investigation without its destruction or interruption of the process studied. Moreover, NMR imaging is based on the principles of nuclear magnetic resonance, and, therefore, its spectroscopic modality can be used to determine a molecular composition of a reaction mixture within an operating biocatalytic reactor.

In the current work, an attempt to apply NMR techniques in order to study biocatalysts and biocatalytic processes was made. A glucose isomerization reaction catalysed by a glucose isomerase enzyme produced by *Arthrobacter* cells immobilized on a silica xerogel was chosen as a model heterogeneous biocatalytic reaction for investigation because of its industrial importance and simplicity. It was shown that the presence of cells in the pores of the support had no influence on the parameters of the ^{1}H NMR signal of a solvent (water), but altered the parameters of the ^{1}H NMR signal of a reagent (glucose) and a product (fructose) allowing us to map the distribution of the active component in the catalyst bed by the NMR imaging technique. It was also shown that ^{13}C NMR spectroscopy can be successfully applied to follow the progress of the glucose isomerization reaction in a batch reactor. The analysis of the ^{13}C NMR spectra of the reaction mixture in different time moments allowed us to determine a mixture composition and estimate a conversion degree. However, the signal to noise ratio (S/N) in the ^{13}C NMR spectra significantly decreased, when the spectra were acquired for a fixed biocatalyst bed filled with the reaction mixture, because of the smaller amount of a liquid phase enclosed in voids between the catalyst particles. An increase of S/N in the ^{13}C NMR spectra is required, if the progress of the glucose isomerization reaction will be followed by ^{13}C NMR spectroscopy in a functioning biocatalytic reactor with a fixed biocatalyst bed and a flow of the reaction mixture *in situ*. It was shown that by utilization of spin decoupling and nuclear Overhauser effects a significant increase of S/N in the ^{13}C NMR spectra of glucose and fructose in a porous matrix can be achieved that allows us to reckon on these techniques, when the glucose isomerization reaction will be studied by NMR in a fixed bed biocatalytic reactor *in situ*.

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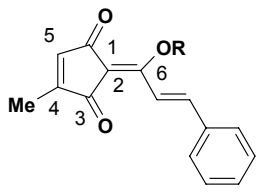
SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF METHYL ENOL ETHERS OF CYCLOPENTENE *β,β′*-TRIKETONES

[1]Martyyas, E.A., [2]Shestak, O.P., [2]Anisimov, M.M., [2]Novikov, V.L.
[1]*Far-Eastern State University, Vladivostok, Oktyabrskaya, 27, fax (4232) 457609*
[2] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*

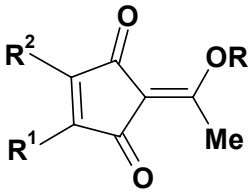
e-mail: shestak@piboc.dvo.ru

In the last few years considerable attention has been focussed on the chemical and pharmacological investigations of natural cyclopentene *β,β′*-triketones and their methyl enol ethers [1, 2]. One of them, coruscanone A (**2**), showed high fungicidal activity against fungi *Candida albicans* and *Cryptococcus neoformans*, two major opportunistic pathogens associated with AIDS patients [1]. On this basis, we initiated the present investigation in other to synthesize a number of methyl enol ethers of 2-acetylcyclopent-4-ene-1,3-diones and to evaluate for their *in vitro* antimicrobial activities.

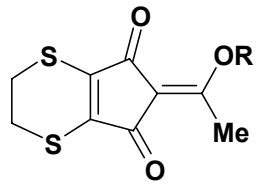
Coruscanone B (**1**) was obtained by the reaction of citraconic anhydride with the corresponding phosphorane and the further rearrangement of prepared 4-ylidenebutenolide under the action of MeONa. Coruscanone A (**2**) was prepared by the methylation of coruscanone B (**1**) with dimethyl sulfate. The starting monocyclic *β,β′*-triketones (**3**)-(**9**) were synthesized by the acylation of isopropenyl acetate with the corresponding maleic anhydrides. The methyl enol ethers (**10**)-(**16**) were obtained through the reactions of free triketones (**3**)-(**9**) with CH_2N_2. Bicyclic *β,β′*-triketones (**17**)-(**19**) and their enol ethers (**20**)-(**22**), respectively, were made also.



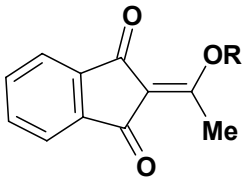
1 R=H; **2** R=Me
mixtures of Z/E isomers
about C(2)-C(6) double bond



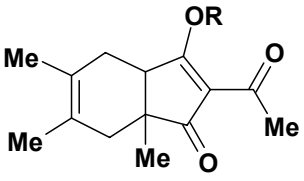
3 R=R^1=R^2=H; **10** R=Me, R^1=R^2=H
4 R=R^2=H, R^1=Me; **11** R=R^1=Me, R^2=H
5 R=H, R^1=R^2=Me; **12** R=R^1=R^2=Me
6 R=H, R^1=R^2=SMe; **13** R=Me, R^1=R^2=SMe
7 R=R^1=H, R^2=Br; **14** R=Me, R^1=Br, R^2=H
8 R=R^1=H, R^2=Cl; **15** R=Me, R^1=Cl, R^2=H
9 R=H, R^1=R^2=Cl; **16** R=Me, R^1=R^2=Cl
(**4**), (**7**), (**8**), (**11**), (**14**), (**15**)–mixtures of Z/E isomers



17 R=H; **20** R=Me



18 R=H; **21** R=Me



19 R=H; **22** R=Me

For biotesting were used Gram positive bacteria *Staphylococcus aureus* and Gram negative bacteria *Escherichia coli*, beer yeast *Sofale* S-04, yeast-like fungi *Candida albicans* and pathogenic fungi *Fusarium oxysporum* and *Aspergillus niger*, when were grown on solid medium. Antimicrobial action *in vitro* of tested compounds was determined by the method of diffusion in agar-agar and evaluated through the width of growth inhibition zone (mm).

Synthetic triketones (**3**) and (**9**) showed high inhibiting action against *S. aureus* at a concentration of 100 μg/ml. Compound (**9**) was active at the same concentration against *E. coli.*

Methyl enol ether (**15**) showed the most activity against beer yeast *Sofale* S-04. Natural methyl ether, coruscanone A (**2**), exhibited the most activity against fungi *C. albicans, F. oxysporum* and *A. niger.* The simplest synthetic analogue of coruscanone A, compound (**11**), was also sufficiently active against *F. oxysporum*, but 3 times less active against *C. albicans.* Ether (**16**) displayed high activity against fungi *A. niger.*

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THE NATURE OF SELF-ORGANIZATION PHENOMENA IN CATALYTIC CO OXIDATION ON Pd(110)

Matveev, A.V., Sametova, A.A., Gorodetskii, V.V.
Boreskov Institute of Catalysis SB RAS, Novosibisk, pr. Ak. Lavrentieva 5, 630090, Russia
fax: +7 383 330 80 56;
e-mail: matveev@catalysis.ru

The self-organization phenomena (hysteresis, oscillations and chemical waves) in $CO+O_2$ reaction on Pd, Pt and Ir were observed for the first time in 80-s by Sales, Turner and Maple [1], who proposed the reversible formation of oxide layer PdO_x in reaction conditions (~ 1 mbar). Afterwards Ladas, Imbihl and Ertl [2] found the unsteady state phenomena on Pd(110) single crystal surface at lower pressures (< 10^{-2} mbar) and deduced that the formation of "subsurface oxygen" species (O_{subs}) was a driving force for reaction rate oscillations. Till now there is still no common agreement in the nature of such unusual phenomena in CO oxidation on Pd(110) [3].

Using the X-ray photoelectronic spectroscopy (XPS) we found, that on a polycrystalline palladium surface in conditions $P(O_2) = 10^{-2}$ mbar, $P(CO) \sim 10^{-6}$ mbar ($P(O_2)/P(CO) \sim 10^4$), T = 525 K, close to conditions of self-oscillation regime on Pd(110), oxide formation did not occur but there was a penetration of oxygen atoms into the top layer of metal and formation of subsurface oxygen layer. Transient kinetic experiments on Pd(110) showed that CO_{ads} molecules did not react chemically with subsurface oxygen below 300 K. O_{ads} atoms are highly active compared to O_{subs} species due to the rapid reaction with CO_{ads} beginning at temperature ~ 150 K. Based on experimental data, the detailed mechanism of CO oxidation on Pd(110) was proposed. During the imitating experiments, carried out by Monte Carlo calculations, the set of parameters has been found where the model showed a self-oscillatory regime. The hysteresis in oscillatory behaviour was observed under step-by-step variation of oxygen partial pressure. Two different oscillatory regimes exist at one and the same parameters of the reaction. The parameters of oscillations (amplitude, period and the shape of the waves on the surface) depend on the kinetic prehistory of the system. The possibility for the appearance of the cellular and turbulent patterns, spiral, rings and stripe oxygen waves on the surface has been shown.

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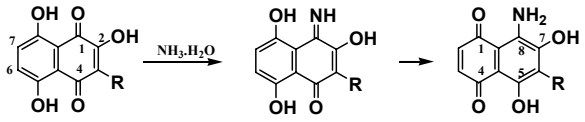
THE REACTION OF ECHINOCHROME TRIMETHYLETHER WITH AMMONIA

Melman, G.I., Anufriev, V.Ph., Denisenko, V.A.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: sopelnyak@piboc.dvo.ru

Recently, we were stated that 2-hydroxynaphthazarins **1**, **2** reacted regiospecifically at the one position with ammonia to provide corresponding 8-aminojuglone derivatives (Scheme 1).[1] This reaction is directed by *β*-OH group at the second position and proceeds *via* an addition – elimination mechanism.[2]

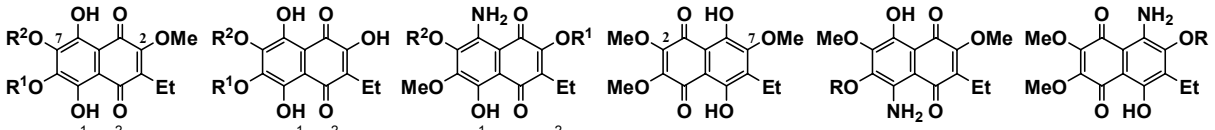


Scheme 1

1, 2 R=H, Et

The certain selectivity in the reaction of polymethoxynaphthazarins with ammonia we observed also. So, it was established, that the reaction of echinochrome trimethylether (ETME, **3**) with a solution of conc. ammonia leads to a mixture of 8-aminojuglones (NMR), in which the one isomer was the main (77%). The available physicochemical methods (NMR, EIMS, IR) do not allow unambiguous conclusions about the assignment signals for the each product. To solve this problem we synthesized hydroxynaphthazarins **4**, **5** and **6**. The mixture of these compounds was synthesized by the acid[3] and alkaline[4] hydrolysis of ETME, the partial methylation of echinochrome A (**7**) on a silica gel.[5] A more acceptable result was obtained when the alkaline hydrolysis of ETME used. The ratio of the products **4**, **5**, and **6** in mixture was 42%, 42% and 6%, respectively. Hydroxynaphthazarins **4-6** reacted with $NH_3 \cdot H_2O$ to provide corresponding 8-aminojuglones **8**, **9**, and **10** in excellent yields.

The structure of these compounds was established by methods of the mass-spectrometry, the NMR ^1H and ^{13}C spectroscopy with attraction of the HMBC data.



3 $R^1=R^2=Me$
4 $R^1=Me$, $R^2=H$
6 $R^1=H$, $R^2=Me$

5 $R^1=R^2=Me$
7 $R^1=R^2=H$

8 $R^1=Me$, $R^2=H$
11 $R^1=R^2=Me$

3a

10 R=H
13 R=Me

9 R=H
12 R=Me

Methylation of aminojuglones **8-10** by CH_2N_2 gives corresponding trimethoxynaphthazarins **11**, **12**, and **13**.

The comparative NMR ^1H analysis showed that the proton signals of trimethyl ether **11** derived from aminojuglone **8** completely agree with the proton signals of the main product obtained by the reaction of ETME **3** (in the tautomeric form **3a**) with $NH_3 \cdot H_2O$.

According to the NMR ^1H data the product **12** is identical to one of minor 8-aminojuglone, obtained by the reaction of the tautomer **3** with $NH_3 \cdot H_2O$. The methylation of the compound **10** leads to aminojuglone trimethyl ether **13**. Among the reaction products of substrate **3** with $NH_3 \cdot H_2O$ the compound **13** it is not discovered.

Thus, reaction of tautomeric forms ETME (**3**, **3a**) with $NH_3 \cdot H_2O$ proceeds at the carbonyl groups located between the methoxy groups at 2,7- positions, mainly at the carbonyl group of tautomer **3a.**

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OXIDATIVE DEHYDROGENATION OF LIGHT HYDROCARBONS OVER AEROGEL VOx/MgO CATALYST

Mishakov, I.V., Vedyagin, A.A., Ilyina, E.V.
Boreskov Institute of catalysis SB RAS, 630090, Novosibirsk, pr. Lavrentieva, 5, Russia
e-mail: vedyagin@catalysis.ru

With increasing oil prices an alternative process for the production of lower alkenes is economically reasonable. Oxidative dehydrogenation (ODH), e.g. of propane, is an attractive reaction route instead of steam cracking of naphtha or catalytic cracking/dehydrogenation. This alternative process is energetically favorable due to lower reaction temperatures and enhanced catalyst lifetime thanks to prevention of the coke deposition. Furthermore, it is neither equilibrium-limited nor endothermic. Therefore, it has important advantages compared to the established processes. Due to the increasing world demand for propene, the ODH reaction is a great challenge for catalysis research and development, and growing attention has been focused on this topic in the past decade.

A considerable amount of work has been devoted to the development of catalysts with good selectivity to olefins in the oxidative dehydrogenation of paraffins. V/MgO was found to be one of the best catalytic systems in terms of activity and performance stability. The reaction is believed to follow a Mars–van Krevelen reaction mechanism, in which adsorbed propane reacts with lattice oxygen and the reduced metal oxide reacts with adsorbed dissociated O_2.

Despite good activity for the ODH of alkanes to their corresponding alkenes, non-selective combustion pathways limit the alkene selectivities, especially at high conversions. Particularly for oxidative dehydrogenation of propane to propene, it has been well established that limited propene selectivity at higher propane conversions is related to propene adsorption on acid sites and their subsequent combustion to carbon oxides. Thus, new efficient catalytic systems that would allow for effective propene production with high selectivity at higher propane conversions are highly desirable.

In the present work, the new approach for the synthesis of nanoscale aerogel VO_x/MgO catalysts has been suggested. In short it includes formation of $Mg(OCH_3)_2$ via reaction of Mg with methanol, dilution with toluene, addition of vanadium isopropoxide, gel formation by hydrolysis, supercritical drying of resultant suspension, and conversion of aerogel V-Mg-hydroxide into VO_x/MgO via heat treatment under vacuum at 500 °C overnight and ultimate calcination in air at 550 °C for several hours. It was found that the developed aerogel technology allowed us to synthesize the mixed V-Mg-hydroxides with a surface area as high as 1000-1300 m^2/g. The interim stage of vacuum dehydration of V-Mg-hydroxides was applied to convert the aerogel V-Mg-hydroxides into VO_x/MgO catalysts with a final surface area of about 350 m^2/g, which is superior to analogues found in literature [1-3].

Aerogel VO_x/MgO catalysts were studied in comparative ODHP tests along with reference samples prepared on the basis of nanoscale MgO-AP support by mechanical mixing and impregnation methods. At all other parameters kept the same, the aerogel VO_x/MgO samples turned out to be significantly more active as compared with similar catalysts prepared by conventional ways. It was also shown that the replacement of O_2 by N_2O in the ODHP reaction gives the considerable benefit in selectivity towards C_3H_6, however the yield of propylene does not change. This is as well accompanied with more intensive process of coke formation on the surface of aerogel VO_x/MgO catalysts.

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DECOMPOSITION OF 1,2-DICHLOROETHANE OVER NICKEL-CONTAINING CATALYST

Mishakov, I.V., Vedyagin, A.A. and Strel'tsov I.A.
Boreskov Institute of catalysis SB RAS, 630090, Novosibirsk, pr. Lavrentieva, 5, Russia
e-mail: mishakov@catalysis.ru

Volatile organic compounds are considered as one of the main air pollutants, either directly through their toxic or malodorous nature, or indirectly as ozone precursors and smog precursors. Besides main emission sources such as petroleum industries, there are a lot of local sources such as painting, printing and laundry which emit VOCs stream of low concentration. Among all VOCs, chlorine-containing compounds require a special attention due to their toxicity, high stability and widespread application in industry [1].

The pyrolysis of 1,2-dichloroethane (1,2-DCE) has been commercialized as a way to produce vinyl chloride monomer (VCM). This process gives a reasonable conversion of 50% and a high selectivity of 98%, both on industrial scale [2]. Thus, the wastes of this process contain a significant amounts of non-converted 1,2-DCE. Catalytic decomposition of chlorinated hydrocarbons over nickel- and cobalt-containing catalysts is considered to be an effective approach for abatement of such wastes [3]. This method may also be applied for utilization of other chlorinated hydrocarbons and their complex mixtures. While performing the process of decomposition, the active metal particles of catalyst produce structured carbon filaments according to mechanism of "carbide cycle" proposed by R. Buyanov and coworkers [4]. Formed carbon material was found to have a very disordered structure and named as feather-like carbon due to its special morphologic characteristics [5, 6].

The catalyst Ni/Al_2O_3 was prepared by precipitation of nickel and aluminum nitrates at a constant pH of the solution. The sediment was washed by distilled water, dried and calcined at 330°C in air. Prior to decomposition of $C_2H_4Cl_2$, the catalyst sample was reduced in hydrogen flow at 500°C for 1 hour. The reduced catalyst contained 85 mass.% Ni and 15 mass.% Al_2O_3.

The process of decomposition of the 1,2-DCE was carried out in two different regimes:

- without addition of hydrogen at $T \geq 550$°C;
- with hydrogen at $T \geq 400$°C.

It was established that the decomposition of 1,2-DCE at temperatures under 550°C in the absence of hydrogen results in rapid deactivation of catalyst. Deactivation of catalyst happens due to process of chlorination of Ni particles by HCl to form bulk $NiCl_2$ which takes place at temperatures lower than 550°C. As temperature is increased to 550°C, the catalyst starts to work stable due to the process of self-clarification of metal surfaces from chlorides forced by interaction with excess hydrogen forming during the reaction.

The addition of hydrogen into reaction gas feed allows one to decrease the temperature at which catalyst continues working without deactivation. It should be however mentioned that the temperature drop along with multiple excess of hydrogen move the catalyst from the "carbide cycle" to the mechanism of hydrodechlorination, where the dechlorinated hydrocarbons (ethane, ethylene and methane) are the main carbon-containing products.

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SEA ANEMONE PORE-FORMING TOXINS: STRUCTURE AND FUNCTION

Monastyrnaya, M.M, Leychenko, E.V, Zelepuga, E.A, Kozlovskaya, E.P

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: rita1950@mail.ru

The α- and β-pore-forming toxins (PFT) produced by different organisms in a soluble form act upon target cell membranes by forming transmembrane channels. They are classified according to the transmembrane structural elements (α-helix ore β-strand) inserted into membrane. Due to conformation movement PFT adapt the hydrophobic parts of their structure for membrane association and pore formation. So, some bacterial β-PFT form stable oligomeric β-barrels which consist from 50 protomers, as α-PFT pores lined by α-helices are many times less therefore they are instable [1]. As a rule all bacterial PFT have a three-domain structure and one domain presents oneself as pore forming.

One entirely novel class of an α-PFT is the sea anemones actinoporins. Unlike bacterial PFT actinoporins possess with peculiar three-dimensional one-domain structure [1]. They are closely related cysteine-less proteins, which exhibit sphingomyelin (SM) dependence. The actinoporin pore forming is a multistep process according to the modern hypothesis. Toxin interacts with the lipid membrane phosphocholin group (POP) by the POP-binding site located on a broad loop at the bottom of molecule and on the C-terminal α-helix. Then an N-terminal α-helix fragment (1-28 AA) translocates to the lipid-water interface and, finally, the helices from four monomers form a transmembrane pore [2].

The structure analysis of POP-binding sites of actinoporins from *Radianthus macrodactylus* [3, 4] and *Oulactis orientalis* [5] as well as some other representatives of Stichodactylidae family shows the presence of Leu instead the Trp^{112} in some of them. This testifies contrarily the affirmation that only Trpresidue in 112 position is crucial for the actinoporin pore forming activity accordingly to date [6]. We determined that considerable differences in values of Radianthus and Oulactis actinoporin's haemolytic activity are conditioned by some discrepancies in N-terminal lieder segments (1-10 AA). The hydrophobicity paramethers of lieder actinoporin segments (<μH> and <H> are calculated by application program HydroMCalc accordingly to Eisenberg scale) are way below than such of long segments, consisted from 10-28 and 16-26 AA. Their mean hydrophobic moment ((<μH>)≥0.3-0.4) is identical with <μH> of antimicrobial peptides and many proteins possessed by N-terminal α-helical fragment.

We suppose that one critical factor for actinoporin's pore forming activity like to such of membrane-active peptides is the electrostatic interaction of N-terminal fragment with membrane interface. There is different number of positive and negative charged amino acids in actinoporins N-terminus. Apparently when positive charges direct a binding with negative charged POP groups of membrane, the location of negative charges in pore lumen is requirement for their high activity.

Considering a little <H> and $<\mu_H>$ values for actinoporins lieder segments as well as existence of several Leu, Gly and Ala in these segments an existence of their α-helix–α-helix interactions may to assume. These interactions resulted in a self association and pore formation of actinoporins. Thus one possible explanation of N-terminus necessity for potency and specificity of actinoporins is that it satisfies to the general criteria for pore forming, such as a positive charge and an amphipathic structure.

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DETERMINATION OF BINDING PARAMETERS OF LIPOPOLYSACCHARIDES WITH CHITOSAN AND ITS N-ACYLATED DERIVATIVES USING PIEZOELECTRIC QUARTZ CRYSTAL SENSOR

Naberezhnykh, G.A., Gorbach, V.I.,Kalmykova, E.N., Ermolaeva, T.N., Solov'eva, T.F.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
E-mail: naber1953r@mail.ru

Lipopolysaccharide (LPS), also known as an endotoxin, is the major outer-membrane constituent of gram-negative which is composed of three distinct regions: the lipid A, core and the O-specific antigen. Lipid A is responsible for many patho-phisiological effects include septic shock, pyrogenicity and multiple-system organ failure. Therefore, to find a drug which can bind to LPS and neutralize its toxicity is very important for clinical therapy. The anionic and amphiphilic nature of lipid A enables it to bind to numerous substances which are positively charged and which also possess an amphipathic character. We have earlier characterized the interactions of LPS with the natural polycation chitosan, oligochitosan (5.5 kD) and it N-monoacylated derivatives using ligand–enzyme solid-phase assay and molecular modeling. The input of the hydrophobic fragment in complex formation energy is most prominent for complexes in water phase and is due to the hydrophobic interaction of chitooligosaccharide acyl fragment with fatty acid residues of LPS.

In this study, we developed a flow injection analysis system using an LPS-coated piezoelectric quartz crystal (PQC) sensor. This technique is minimally invasive to the binding, requires no labeling, and real-time measurement kinetic and equilibrium constants with low sample volumes. The amino group-modified gold surface PQC was activated by the glutaraldehyde and LPS was immobilized on the crystal. It is well known that the resonant frequency of an oscillating crystal can be affected by an instant change mass of ligand-receptor complex. Therefore, by comparing maximum of frequency decrease of different ligand one can compare their activity. The LPS-modified PQC gave 15-20 Hz frequency change at minimal N-monoacylated oligochitosan concentration of 2×10^{-7} M. It was shown that LPS-binding activity of N-monoacylated oligochitosan was greater about 1,5 times that of oligochitosan.

The method involves measuring the rate of the frequency shift proportional to the amount of chitosan added was used to estimate association (k_a) and dissociation constants (k_d) for reaction of LPS with oligochitosan or N-monoacylated oligochitosan. In the range concentration of 2×10^{-7} - 2×10^{-6} M, the k_a obtains are 1.27×10^4 and 1.04×10^4 M^{-1} s^{-1}. k_d estimate are 3.8×10^{-2} and 3.11×10^{-2} s^{-1}, respectively. The equilibrium constant $K = k_a/k_d$ of reaction between LPS with oligochitosan or N-monoacylated oligochitosan are 3.13×10^5 and 3.54×10^5 M^{-1}, respectively. The K values were determined for oligochitosan specimens have similarity. This is near the K determined earlier for complex LPS with chitosan (15 kDa) by other methods. The present study suggest the PQC biosensor is potentially useful for the detection and comparison of the LPS-binding ability of different chitosan derivation by using an LPS-coated piezoelectric crystal.

WINOGRADSKYELLA ECHINORUM SP. NOV., A NOVEL MARINE BACTERIUM OF THE FAMILY *FLAVOBACTERIACEAE*

Nedashkovskaya, O.I. [1], Vancanneyt,M. [2], Kim, S.B. [3], Zhukova, N.V. [4]

[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*
[2] *BCCM/LMG Bacteria Collection and Laboratory of Microbiology, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium*
[3] *Department of Microbiology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yuseong, Daejeon 305-764, Republic of Korea*
[4] *Institute of Marine Biology of the Far-Eastern Branch of the Russian Academy of Sciences, Pal'chevskogo St. 17, 690032, Vladivostok, Russia*

e-mail: olganedashkovska@piboc.dvo.ru or olganedashkovska@yahoo.com

The genus *Winogradskyella,* a member of the family *Flavobacteriaceae*, was created to accommodate heterotrophic, aerobic, yellow-pigmented, Gram-negative and motile by gliding bacteria (Nedashkovskaya *et al*., 2005). Currently, the genus comprises four recognized species, *W. epiphytica, W. eximia, W. poriferorum* and *W. thalassocola* (Lau *et al*., 2005; Nedashkovskaya *et al.,* 2005). The type strains of the genus *Winogradskyella* were isolated from the green alga *Acrosiphonia sonderi*, from the brown algae *Chorda filum* and *Laminaria japonica* collected in the East Sea and from sponge *Lissodendoryx isodictyalis* inhabited near Bahamas.In the course of study of taxonomic diversity within microbial community of the sea urchin *Strongylocentrotus intermedius* the precise taxonomic position of a novel marine yellow-pigmented bacterium, designed strain KMM 6211^T, was examined using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain KMM 6211^T is a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes*. A closest relative of the strain studied was *Winogradskyella eximia* KMM 3944^T with sequence similarity of 97.1%. The DNA G + C content of KMM 6211^T was 33.6 mol%. Phosphatidylethanolamine was the only phospholipid identified. The predominant cellular fatty acids of strain KMM 6211^T were straight-chain unsaturated, branched-chain unsaturated and saturated, namely iso-$C_{15:1,}$ iso-$C_{15:0}$, summed feature 3 (consisting of iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{15:0}$, iso-$C_{15:0}$ 3-OH and iso-$C_{17:0}$ 3-OH. These values are in consistence with those reported for recognized members of the genus *Winogradskyella*. The strain grew with 1-6% NaCl and at 4-37°C. Aesculin, casein and gelatin were hydrolyzed but agar, starch, DNA and chitin were not decomposed. Like other *Winogradskyella* species, the novel isolate possesses oxidase, catalase, alkaline phosphatase and gelatinase activities, requires Na^+ ions for growth and moves by gliding. However, strain KMM 6211^T is characterized by the presence of β-galactosidase activity and by the absence of esterase (Tween 40) production in contrast with published members of the genus *Winogradskyella*. Strain KMM 6211^T clearly differs from its closest phylogenetic relative, *Winogradskyella eximia* KMM 3944^T, in terms of hydrolysis of agar and starch, growth at 37°C, acid production from D-glucose, maltose, sucrose and mannitol, and utilization of sucrose and mannitol A phylogenetic evidence taken together with differences in phenotypic characteristics found between strain KMM 6211^T and recognized *Winogradskyella* species justifies a creation of a new species, for which the name *Winogradskyella echinorum* sp. nov. is proposed. The type strain is KMM 6211^T (= KCTC 22026^T = LMG 30325^T).

CONFOMATIONAL PLASTICITY OF OMPF-LIKE PORIN FROM *YERSINIA PSEUDOTUBERCULOSIS*

Novikova, O.D., Khomenko, V.A., Kim, N.Yu..
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: novolga_05@mail.ru

One of the major physico-chemical characteristics of proteins is their resistance to the action of different denaturants. According to contemporary presentation the separate structural regions of porin molecule are distinguished in stability. This circumstance predetermines the multiplicity of denatured states of these oligomeric proteins in different denatured conditions. Structure and function of porin conformational intermediates have been studied weakly. However, these data are necessary for revealing structure-functional changes in porin molecule under denaturation and for understanding molecular bases of porin folding.

Using methods of SDS, PAGE-electrophoresis, optical spectroscopy (intrinsic protein fluorescence, CD spectroscopy and fluorescence emission spectroscopy of the added hydrophobic fluorescence probes), scanning microcalorimetry, and reconstitution in bilayer lipid membrane (BLM), the pH-dependence of structure and functional activity of Omp F-like porin from outer membrane (OM) of *Yersinia pseudotuberculosis* (yersinin) were studied in the pH range of 7.5.-2.0. pH-Induced conformational intermediates of yersinin are examined in terms using for characterization of water-soluble proteins.

Mechanism of yersinin pH unfolding may be described by three state-model: (1) disordering of porin associates with formation of native-like porin trimers — (2) independent domain unfolding with subsequent cooperative dissociating of trimers — (3) formation of two loosly structured forms of monomer intermediates. First of monomers (at pH 3.0) appeared to be molten globule-like state of porin with native-like secondary structure. The second one (at pH 2.0) was shown to be misfolded monomer, which retains only 50% of regular secondary structure. Using theoretical model of yersinin, possible mechanism of β-barrel unfolding under pH has been discussed.

The analysis of a current fluctuation in BLM showed that the magnitude of pore conductivity of yersinin in acidic pH range decreased by the order. The sharpest change in activity of the protein was marked at pH 5.8, whereas the transition of channels in the closed state was observed at pH 5.0. Such sharp decrease of pore-forming activity of trimeric form of yersinin observed in this narrow range of pH, probably, precede the start of the common mechanism of bacteria adaptation to change of external conditions. The last is known to be connected with the regulation of non-specific porin biosynthesis. The interrelation observed between changes in yersinin structure and its functional activity under pH can serve as the obvious experimental proof of conformational and functional plasticity of porins.

A specific interaction between lipopolysaccharide (LPS) and porin in the native bacterial membrane determines the OM properties and is crucial for the life of the bacterial cell. Only interacting to LPS, porin oligomerizes and reconstitutes as a mature protein to the OM. Probably, the conformation of the mature porin that determines its insertion into the OM. However, it is still unclear whether or not a prefolded by LPS protein intermediate really exists *in vivo*.

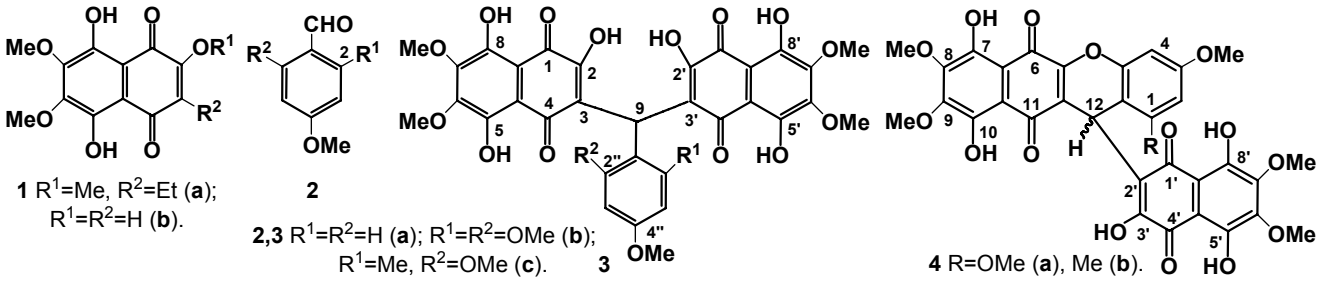
Changes in the structure and functional activity of the porin, resulting from the removal of LPS, normally bound with the protein were studied. According the data of SDS, PAGE, LPS-free porin retained a trimer. However, the certain conformational changes in the spatial structure of the protein have been revealed using CD and UV spectroscopies and intrinsic protein fluorescence. LPS-free protein folded into a completely β-structured protein aggregate. The BLM technique showed that the pore-forming activity of the LPS-free porin decreased, and its concentration should be increased by two orders of magnitude to achieve the same effect. Incubation of the LPS-free porin with the LPS led to a porin-LPS complex and affected the character of the protein functional activity. The treatment of the LPS-free porin by octyl glucoside, a nonionic detergent, resulted in the appearance of non-regular structure in the porin molecule and the restoration of the protein pore-forming activity. It was suggested that the LPS and detergent provide a definite protein conformation necessary for its function.

SYNTHESIS OF BENZYLIDENEBISNAPHTHAZARINS - STRUCTURAL ANALOGUES OF SOME SEA URCHINS METABOLITES

Pelageev, D.N., Panchenko, M.N., Anufriev, V.Ph.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: pelageev@piboc.dvo.ru

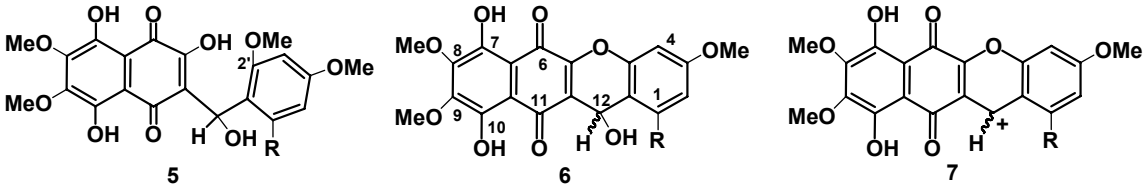
The chemistry of polymethoxynaphthazarins (5,8 dihydroxy-1,4-naphthoquinones) is poorly studied.[1] We have devised convenient methods for obtaining these compounds[2] what makes them attractive as starting substances in the synthesis of a number of natural products and their analogues.[3] So we have worked out a sufficiently simple way of conversion of echinochrome trimethyl ether **1a** into spinochrome D dimethyl ether **1b**, from which one of pigments of sea urchins *Strongylocentrotus intermedius* and *S. dröebachiensis* was synthesized.

During these studies we examined the reaction of dimethyl ether **1b** with aromatic aldehydes **2a-c**. So in the present of $Et_3N{\cdot}HCl$ the reaction of **1b** with anisaldehyde (**2a**) gave tetramethyl ether of arylidenbisnaphthazarin **3a** in high yield.



1 R^1=Me, R^2=Et (**a**); R^1=R^2=H (**b**). **2** **2,3** R^1=R^2=H (**a**); R^1=R^2=OMe (**b**); R^1=Me, R^2=OMe (**c**). **3** **4** R=OMe (**a**), Me (**b**).

On the other hand the reaction of substance **1b** with 2,4,6-trimethoxy- (**2b**) and 2,4-dimethoxy-6-methylbenzaldehyde (**2c**) gave corresponding benzylidenbisnaphthazarins **3b** and **3c** in poor yield (20-25%). In this case piranonaphthazarins **4a** and **4b** were the major products. Formation of the products **4a,b** is due to ease of 2′-methoxy group hydrolysis in the adduct **5a,b** and cyclization of resulting 2′-hydroxy derivative into corresponding xanthydrols **6a,b**.



5 **6** **7**

5,6,7 R=OMe (**a**), Me (**b**).

Xanthydrols **6a,b** on their own were not found in reaction mixture that is likely to be by virtue of high xanthydrols reactivity in acid medium. In principle, they can readily give the xanthilic cations of type **7a,b** in the reaction conditions. After these cations attack substrate molecules **1b** the products **4a,b** are formed.

Methoxy group hydrolysis in the position 2′′ of benzylidenbisnaphthazarins and following cyclization of resulting 2′′-hydroxy derivative may be the alternative mechanism.

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EVALUATION OF PARAMETERS OF RESPIRATORY FUNCTION IN PATIENTS WITH CIRRHOSIS ASSOCIATED WITH CHRONIC OBSTRUCTIVE LUNG DISEASE.

[1]Pestrikova, T, [1]Nevzorova, V, [1]Kochetkova, E, [2]Oswald-Mammasser, M
[1]*Vladivostok State Medical University, Ostryakova Prospekt, 2, Vladivostok 690600, Russia*
[2] *University of Louis Pasteur, Kirschleger Street, 4, Strasburg 67085, France,*
e-mail: pestriktl@mail.ru

Hepatopulmonary syndrome (HPS) is characterized by clinical triad such as chronic liver disease and increase of alveoloarterial oxygen difference ($AaDO_2$) and intrapulmonary vascular dilatation.

The aime our study to evaluate the features of hepatopulmonary syndrome in patients with cirrhosis and in patients with cirrhosis associated with chronic obstructive pulmonary disease (COPD).

We carried out retrospective analysis of cases history for 75 patients with cirrhosis different etiology. The patients were divided into two groups: first (n = 23) – patients with cirrhosis and COPD, and second (n = 52) – with cirrhosis. The patients of both groups were with similar age. Spirography, gasometry, single-breath carbon monoxide diffusion capacity (DL_{co}) and exhaled nitric oxide (NO) production were observed. Transthoracic contrast-enhanced echocardiography was performed in case of increase $AaDO_2 \geq 15$ mm Hg for detecting intrapulmonary shunt (IPS).

In 8 of the 23 patients had mild COPD and 14 patients had moderate COPD. 12 Patients were smokers; 26.4 ± 17.7 pack/years. Oxyhemoglobin (HbCO) was not significant different among groups (2.19 ± 1.1% и 2.26 ± 1.2 %, $p > 0.05$, accordingly). DL_{co} had tendency to increase in group 1 ($p > 0,05$). PaO_2 was decreased in group 1 (83.2 ± 6.2 mm Hg and 96.3 ± 4.3 mm Hg, $p < 0.05$) and $AaDO_2$ was increased in group 1 (25.8 ± 4.3 mm Hg and 14.5 ± 5.3 mm Hg, $p < 0.05$). Intrapulmonary shunts were detected in 3 patients of both groups. Concentrations of exhaled nitric oxide (NO) was higher too in group 1 (22.8 ± 10.5 ppb (part per billion) and 20.3 ± 12.1 ppb), but difference was not significant ($p > 0.05$).

Different pathologies: cirrhosis and COPD not exclude the development of two different reactions (vasodilatation and vasoconstriction). We determined that exist the tendency of more expressed hypoxemia in cirrhosis patients with COPD than in cirrhosis patients without COPD. One can suppose that the production of inhibitors of vasoconstrictors is increased in cirrhosis associated with COPD in stage of respiratory insufficiency.

IMMUNOACTIVE SYNTETIC FRAGMENTS OF OUTER MEMBRANE PORINS OF PATHOGENIC *YERSINIA*.

Portnyagina, O. Yu., Sidorova, O.V., Novikova, O.D.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: odd64@mail.ru

Study on the antigenic determinant structures of OmpC- and OmpF-like porins of pathogenic species of *Yersinia*: *Y. pseudotuberculosis* and *Y. enterocolitica* is one of the main themes of the Laboratory of the molecular basis of antibacterial immunity of PIBOC. Special attention has been paid to investigation of the conservative peptides that possess with the wide spectrum of specificity to different alleles of human lymphocytes. Using various computer programs, overall antigenic-active regions in the primary structure of pathogenic *Yersinia* porins, maximally accessible for interaction with the antibodies, and therefore most promising for inducting the protective humoral immune response were determined. The multivalent antigenic peptides (MAPs) whose sequences include the antigenic epitopes of outer membrane (OM) of *Y. pseudotuberculosis* and *Y. enterocolitica* porins have been synthesized on the basis of calculation data. MAP-1 and MAP-2 are corresponding to sequence regions of *Y. pseudotuberculosis* and *Y. enterocolitica* OmpC porins; MAP-3 and MAP-4 are corresponding to sequence regions of *Y. pseudotuberculosis* OmpF porin.

The synthesized peptides containing B - epitopes were checked up on the ability to connect with the immunoglobulins of the sera with intestinal yersiniosis and pseudotuberculosis. All MAPs were found to react with the blood sera of patients in diagnostic titer (1/800). MAP -1 and MAP-2 interacted with the antibodies specific both to *Y. pseudotuberculosis* and *Y. enterocolitica*. However, in this case amount of antibodies to MAP-1 was revealed in 2.5 times more than antibodies to MAP-2. MAP-3 and MAP-4, corresponding to the epitopes of *Y. pseudotuberculosis* OmpF porin also interacted with the sera of the patients with pseudotuberculosis; but MAP-3 connected with the antibodies in 1.3 times more effective than MAP-4. The results obtained show that all synthetic MAPs contain the antigenic sites in different degree corresponding to B-epitopes of *Y. pseudotuberculosis* and *Y. enterocolitica* porins.

Immune serum was obtained after immunization of animals (BALB/c mouse) by synthetic peptides in the mixture with the complete Freund's adjuvant. The significant titers of the specific antibodies (titer in - lg 3.55) were discovered only in the serum to MAP -1. In spite of this all sera interacted in ELISA with OmpC- and OmpF- like porins, isolated from OM the of *Y. pseudotuberculosis*, grown at different temperatures, at 37° and 4°C accordingly. Antibodies to MAP - 1 were shown to interact more effectively with OmpC porin, than antibodies to MAP – 2. Antibodies to MAP -3 were more effective in reaction with OmpF porin, in comparison with the antibodies to MAP - 4.

It was found that only MAP -1 induces the development of significant level of humoral immune response in mice and the greatest quantity of antibodies in the blood serum of patients to it is revealed also. It is possible to assert that only this peptide contains the valuable antigenic sites which activate both the T- helper and B-cells. Since the MAP -1 contains the motives of binding with the human antigens of the main complex of the histocompatibility of the II class, it can be assumed that this peptide will manifest activity in the composition of the vaccine preparation, intended for the protection of people from the infections, caused by *Y. pseudotuberculosis* and *Y. enterocolitica.*

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YERSINIA PATHOADAPTATION; ENDLESS CHOICE BETWEEN INSECTS AND MAMMALS

Rakin A.V.

Max von Pettenkofer-Institut, LMU, Pettenkoferstr. 9a, 80336 Munich, Germany

rakin@mvp.uni-muenchen.de

Yersiniae comprise an important group of bacterial pathogens, with *Y.enterocolitica*, Y. *pseudotuberculosis* and *Y. pestis* representing the major species of interest. *Y. pestis* is the etiological agent of plague, while *Y.pseudotuberculosis* and *Y. enterocolitica* are enteropathogens that cause a broad range of gastrointestinal syndromes ranging from acute gastroenteritis to mesenteric lymphadenitis. A recently acquired blood-borne transmission by a fleabite distinguishes *Y. pestis* from closely related *Y. pseudotuberculosis*. Just a few discrete genetic leaps are suspected to be sufficient to give rise to a new flea-borne transmission. Acquisition of two *Y. pestis*-specific plasmids and recruitment of endogenous chromosomal genes for flea-associated functions might be crucial for modern plague agent appearance. Sixteen *Y. pestis*, three *Y. pseudotuberculosis*, and single *Y. enterocolitica* genomes that are now in the sequencing pipeline, partially or completely finished, and whole genome microarray hybridization experiments present a slice of an on-going process of *Y. pestis* pathoadaptation to a newly acquired life style. However, presence of functional insect-associated genes not only in closely related *Y. pseudotuberculosis* but also in distant *Y. enterocolitica* relative supports “dual-host” nature of Yersinia that always have to balance between cold and warm blooded hosts. Multiple gene acquisitions with parallel enormous gene reduction and inactivation of the no longer necessary “avirulent” genes are the hallmarks of this on-going pathoadaptation process.

HYDROLYTIC ENZYMES OF MARINE ORGANISMS AS NEW TOOLS FOR MEDICINE AND BIOTECHNOLOGY

Rasskazov,V.A., Balabanova, L.A, Bakunina, I.Yu, Kozhemyako, V.B., Kovalchuk,S.N., Lichatskaya,G.N., Menzorova,N.I., Sibirtzev, Yu.T., Zvyagintseva, T.N.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: raskaz@piboc.dvo.ru

At present the urgency of marine biotechnology for reasonable using biological riches of ocean is rising. Studies of marine organisms as a source of unique medicinal compounds and valuable biochemical preparations (enzymes, pigments, lipids, etc.) in various areas of the industry and agriculture are of a great importance.

The progress in the studies of the hydrolytic enzymes of marine organisms is determined by their exclusive, important role in marine life. Their ability to hydrolyse such cellular biopolymers as polysaccharides, proteins, and nucleic acids determines not only their regulator function in the cellular metabolic pathways, but directly implications of these enzymes in a process of utilization of exogenic biopolymers by different communities of marine organisms. Another reason for interest of the biotechnologists to these enzymes is related with the peculiarities of the inhabiting conditions of the marine organisms. Actually the marine organisms have been recognized to be more adapted to a cold environment in comparison with the terrestrial organisms that in its order is resulted to the cold adaptation of their enzymatic apparatus. Particular features by cold-adapted enzymes appear to be their higher activity at low temperature, easy inactivation by moderate raise in temperature and generally higher catalytic efficiency. Undoubtly these properties of the hydrolases of the marine origin made them more attractive for their using as new catalysts for different biotechnological processes.

One of the important conditions for the practical application of these enzymes has been recognized as accessibility and low cost of sources for their isolation. From this point of view marine organisms seem to be a very promising source of enzymes production because they are reproduced easily by maricultural methods. The possible utilization of the maricultural and fishery industrial wastes for valuable enzyme production may have some economical benefits for both processes.

The systematic investigations of hydrolytic enzymes isolated from marine invertebrates have been realized by the enzymologists of the Pacific Institute of Bioorganic Chemistry of the Far East Division of the Russian Academy of Sciences (PIBOC). Such enzymes of the marine invertebrates as nucleases, phosphatases and 1,3-(beta)-glucanases have been isolated and their properties and specificity have been studied. The results obtained are demonstrated that these enzymes are distinguished from the same type of hydrolases isolated from other sources. Some new enzymes promising for biotechnology such as some nucleases from hepatopancreas of Kamchatian crab and from sea-urchin eggs and embryos as well as highly active alkaline phosphatases from the marine bacteria *Cobetia marina* as well as glucanases isolated from crystalline style of some Bivalvia have been identified. In this report we present some data on properties, action mechanism, and specificity of these enzymes as well as on some aspects of their practical application.

These investigations results have expanded the modern views of molecular mechanisms function of this type of hydrolases as well as its possible role in marine life. The identification of new hydrolytic enzymes with unique properties and specificity among the substances isolated from marine invertebrates presents the new possibilities for their application in biotechnology and medicine.

EVIDENCE OF AMPHIPOD DIET FROM COMBINED FATTY ACID AND STABLE ISOTOPE ANALYSES

Rodkina S., Kiyashko S., Demchenko N., Fadeyev V.
A.V. Zhirmunsky Institute of Marine Biology FEB RAS
e-mail: srokina@mail.ru

Shallow shelf of the north-eastern coast of Sakhalin Island (the Sea of Okhotsk) is characterized by high abundance of benthic invertebrates. Amphipods are the main component of the benthic community in this region and are basic food source for the western population of gray whales. However, what kinds of food sources allow to maintain high amphipod biomass is still unclear. In this work, investigation of food sources of two amphipod species (*Ampelisca eschrichti*, *Anonyx nugax*) from this region was performed using combined fatty acid and stable isotope analyses.

The carbon isotope ratios ($\delta^{13}C$ values) of both species are similar: -18.3 ± 0.2‰ in *A. eschrichti* and 17.5 ± 0.2 ‰ in *A. nugax*. Displaying such a low isotopic ratios, these amphipods could feed either on phytoplankton solely or using partly detritus of terrestrial vascular plants. The average nitrogen isotope ratios ($\delta^{15}N$ values) range from 7.7 ± 0.3‰ in *A. eschrichti* to 12.9 ± 0.4 in *A. nugax*, indicating low trophic level for *A. eschrichti* (primary consumer) and higher trophic status for *A. nugax*. Therefore, *A. eschrichti* feeds on plant food whereas *A. nugax* receives organic matter of primary producer through additional link and probably feeds on animal food.

The fatty acid (FA) compositions of the two amphipod species have been determined by gas chromatography/mass spectrometry. GC analysis of the total FAs of amphipods indicated than more 50 components were present. Polyunsaturated FAs were dominated in *A. eschrichti* (49.8 ± 1.1%), whereas monounsaturated FAs were most abundant in *A. nugax* (50.9 ± 1.4%). Proportion of saturated acids in the total FAs of both species was similar. The saturated FAs were mainly 14:0, 16:0, 18:0 and 3,7,11,15-Me_4-16:0 with small amount of other straight-chain and branched FAs. Proportion of *iso* / *anteiso* branched acids in both species was 0.7-0.6% indicating that bacterial food make negligible contribution on feeding of these amphipod species. The very long chain saturated FAs (marker of vascular plant) were not detected in samples, indicating that there is no terrestrial organic input to these amphipod foods in this region. The most abundant monounsaturated FAs were 16:1n-7 for *A. eschrichti* and 18:1n-9 for *A. nugax*. Proportion of C20,22 monounsaturated FAs in both species was 2.7-2.3%, indicating that copepods also make small contribution on these amphipod species feeding. The main polyunsaturated acid was 20:5n-3 in both species, but higher percentage was in *A. eschrichti* (27.7 ± 0.7%). Other principal polyunsaturated FAs were C16, C18 with higher percentage in *A. eschrichti* and 22:6n-3 with higher percentage in *A. nugax*.

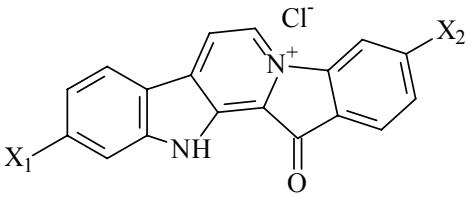
The high percentages of 20:5n-3, 16:1n-7, and sum C16 polyunsaturated FAs together with the ratio 16:1n-7/16:0 > 1 are considered as diatom markers. Total set of diatom markers is presented in *A. eschrichti*, confirming isotopic data supposed herbivorous feeding of this species and making clear diatom phytoplankton origin of its food. The high percentages of 18:1n-9, 22:6n-3, and sum C20, 22 monounsaturated FAs together with the ratio 18:1n-9/18:1n-7 > 1 are considered as markers of animal food. Profile of *A. nugax* FAs shows only high percentage of 18:1n-9 and 18:1n-9/18:1n-7 > 1 and is typical for predatory/scavenger. However, low percentage of sum C20, 22 monounsaturated FAs indicates that copepods are not its main food. Judging from low portion of bacterial FAs, *A. nugax* can not be classified as scavenger. According to data of fatty acid and stable isotope analysis combined, we have suggested that: (1) *A. eschrichti* is suspension feeder and its main food is planktonic diatom; (2) *A. nugax* is carnivorous, and probably its main food is other crustacean like Cumacea or small amphipods, but is not callonoid copepods.

ANTICANCER ACTIVITY OF FASCAPLYSIN AND ITS BROMINATED DERIVATIVES

Savina, A. S.,[1] Fedorov, S. N.,[1] Zhidkov, M. E.,[2] Radchenko, O. S.,[1] Kwak, J. Y.,[3] Stonik, V. A.[1]

[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*
[2]*Far-Eastern National University, Vladivostok, Russian Federation*
[3] *Medical Research Center for Cancer Molecular Therapy, Busan, South Korea*
e-mail: assavina@mail.ru

Alkaloids of fascaplysin type isolated from sponges of *Fascaplysinopsis* genus and ascidia of *Didemnum* genus possess various biological activities including antibacterial, antimalarial, fungicidal, antiviral, and antitumor activity. We here studied anticancer activity of fascaplysin (**1**) and its 3-bromo- and 10-bromo- derivatives (**2** and **3**) obtained by the synthesis.



1: fascaplysin X_1= H, X_2 = H
2: 3-Br-fascaplysin X_1= H, X_2 = Br
3: 10- Br-fascaplysin X_1 = Br, X_2=H

Cytotoxic anticancer or cancer preventive activities of the alkaloids were studied by the MTS or soft agar methods, correspondingly. Method of flow cytometry and luciferase method of the study of a nuclear factor-dependent transcriptional activity were used to investigate the mechanism of the anticancer action of the alkaloids **2** and **3**.

The obtained results indicated that IC_{50} of fascaplysin (**1**) and 3-Br-fascaplysin (**2**) against various human cancer cell lines vary from 200 to 600 nM concentration. 3-Br-fascaplysin (**2**) demonstrated the strongest cytotoxicity, with IC_{50} = 211 nM, in relation to HeLa cancer cells among all six human cancer cell lines studied. Furthermore, 3-Br-fascaplysin (**2**) showed more strong anticancer cytotoxic action than fascaplysin (**1**). The study of cancer preventive activity of 3-Br-fascaplysin (**2**) showed that this alkaloid prevents EGF-induced malignant transformation of JB6 Cl 41 P^+ cells or inhibits phenotype expression of various cancer cell lines at concentrations 1.2 – 7 times lesser than corresponding cytotoxic concentrations.

As was shown by the method of flow cytometry, the anticancer action of 3-Br- or 10- Br-fascaplysin (**2** or **3**), at least in part, can be explained by the induction of apoptosis. Furthermore, it was shown by means of the luciferase method using JB6 Cl 41 p53 cells that alkaloids **2** or **3** inhibited p53-dependent transcriptional activity. Therefore, very likely, apoptosis induced by the compounds **2** or **3** is independent of p53 transcription factor and probably that is why 3-Br-fascaplysin (**2)** showed high anticancer cytotoxic and preventive activity against p53-deficient HL-60 cells.

Thus, brominated derivatives of fascaplysin are perspective as preventive or therapeutic anticancer drugs.

PACIFIC INSTITUTE OF BIOORGANIC CHEMISTRY: INTERNATIONAL COLLABORATION

Natalie M. Shepetova

Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok-22, Russia

Marine natural compounds attract attention of the researchers working in the field of bioorganic chemistry, comparative biochemistry, organic synthesis, and medicine. These compounds are interesting by their structure and biogenesis; many of them show high physiological activity and have important bio-ecological functions. Multipurpose studies of these compounds are of great importance for the scientists over the World.

More than 40 years Pacific Institute of Bioorganic Chemistry is engaged in the comprehensive investigations of biochemical variety of marine algae, macro- and microorganisms. These investigations include collection and identification of macro- and microorganisms to search for new sources of biologically active metabolites, as well as isolation of these metabolites, elucidation of their structure, and development of new medical preparations. Our scientific collaboration in this field with the relative institutes from Australia, Canada, China, Germany, Italy, France, Japan, the Netherlands, New Zealand, U.S.A., South Korea, Vietnam, and the others has resulted in numerous joint articles, patents, and projects. During last ten years more than 30 Agreements about scientific collaboration were signed. Joint marine expeditions aboard the research vessels in the various zones of the World Ocean also play an important role in our relationships. Since 1978 our Institute organized about 40 international marine expeditions. Every year our scientists participate in the international symposia and conferences.

EFFECT OF CULTURE MEDIUM COMPOSITION ON ANTIMICROBIAL ACTIVITY OF MARINE STREPTOMYCETES

Shevchenko, L.S.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: lshev@piboc.dvo.ru

The study of ability to produce bioactive products by some strains of marine streptomycetes grown with various NaCl contents in media was carried out.

A total 12 bacterial strains of *Streptomyces* sp. were used in this work. Out of these, 10 isolates were collected from some samples of sediments in Troitsa Bay (Posyet Bay, Sea of Japan) and 2 strains were isolated from surface of brown alga *Fucus evanescens* (Kuril islands).

All streptomycetes were cultivated in peptone-yeast-glucose agar medium prepared on distilled water with addition of sodium chloride (%): 0.0, 1.0, 3.0, 6.0, 8.0. The antimicrobial activities of each wild strain against *Staphylococcus aureus* and *Candida albicans* were tested using the disc method.

It was appeared that all strains of streptomycetes were tolerant to NaCl and had rich harvest in all media. Out of 12 strains six isolates showed antimicrobial action: five – against *C. albicans*, one – against *S. aureus* and one was found antagonistic to both test-cultures. Streptomycetes could produce bioactive compounds without any salt addition to the medium just as well in the presence of NaCl till 8%.

The metabolites of the strains grown in conditions of high salinity (6.0-8.0 %) displayed the higher inhibitory effect then of those grown in conditions of low or moderate salinity. It is supposed that the ions of NaCl are able to act on the synthesis of biologically active substances under the high saline tensity.

This study was financially supported by the Russian Foundation for Basic Research (Project № 05-04-48211) and Federal Agency for Science of the Russian Federation (Programs “Scientific schools” and “Collection of Marine Microorganisms KMM”) and by grant of FEB RAS № 06-III-A-06-183.

Anticancer effect of 15d-PGJ$_2$ and Stichoposides

Sung-Won Shin,[1,2] Valeria V. Shastina,[2] Valentin A. Stonik,[3] Jong-Yong Kwak,[1,2] Joo-In Park[1,2]

[1]Department of Biochemistry and [2]Medical Research Center for Cancer Molecular Therapy, Dong-A University College of Medicine, Busan 602-714, Korea, [3]Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok 690022, Russia

Recent studies showed that 15-deoxy-$\Delta^{12,\ 14}$-prostaglandin J_2 (15d-PGJ$_2$), a natural ligand for PPARγ, inhibits cell proliferation and induces apoptosis. It was demonstrated that triterpene glycosides from sea cucumbers (holothurians) have a wide spectrum of biological effects: antifungal, antitumor, hemolytic, cytostatic and immunomodulatory activities. Antitumor action of the triterpene glycosides of sea cucumbers was discovered. However, the specific molecular mechanisms underlying these antitumor effects remain to be elucidated. We showed that 15d-PGJ$_2$ induces apoptosis in human leukemia cells in a dose-dependent manner and leads to generation of reactive oxygen species (ROS) and inactivation of Akt and confirmed the antitumor effect of 15d-PGJ$_2$ using CT-26 mouse cancer model. In addition, we clarified the molecular mechanisms for stichoposides-induced apoptosis, and confirmed the anti-cancer effect of these agents in CT-26 mouse cancer model. Thus, these data suggest that these agents may have therapeutic relevance in the treatment of human cancer.

ANTIGENIC PROPERTIES OF THE RECOMBINANT OMPF-LIKE PORIN OF *YERSINIA PSEUDOTUBERCULOSIS*

Sidorova, O, Portnyagina, O., Khomenko, V., Novikova, O.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: olga_ximik@mail.ru

The pore-forming protein (OmpF-like porin) from the outer membrane (OM) of *Yersinia pseudotuberculosis* was expressed via the transformation of the gene coding sequence of this protein in *E. coli* cells Refolding of the recombinant protein (RP), extracted from the inclusion bodies, made it possible to obtain functionally active porin in the monomeric and oligomeric forms.

It is known that porins relate to the highly immunogenic components of the bacterial OM. Immune response to porins is characterized by the high level of the antibodies, obtained after both artificial immunization and spontaneous infection. It was shown that immunization with recombinant porin in the purified form and in the mixture with the Freund's adjuvant, and by the detergents, used for the isolation of protein (Zwittergent 3-14, octyl-β,D-glucopyranoside) initiated in animals (CBA mice) the development of humoral immune response. Mice were immunized with 100 μg RP in the above preparations on days 0.7 and 14. Mice were terminally bled in a week after the last injection. As result, specific high-avidity antiserum against RP (with the titres of antibodies from 1.9 to 3.55, in -lg) were obtained. It was also established that the immunization with RP stimulated the factors of the immediate organism protection, which was illustrated by a significant increase (2.5 times) of the oxygen-dependant bactericidal activity of the peritoneal macrophages of immunized animals in comparison with intact mice.

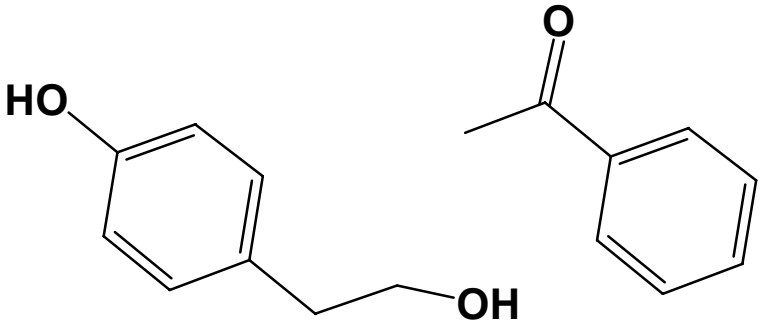
Antigenic structure of monomer and oligomer of the RP were characterized by ELISA with rabbits antisera obtained after immunization with the native monomer and trimer of porin, solubilized from the *Y. pseudotuberculosis* OM. In neither case, it was observed the complete binding of the corresponding specific antibodies to RP. A similar result was also observed with the reaction of the antibodies obtained after mice RP immunization with the native porin. Thus, we made a conclusion that the structure of the antigenic determinants of refolded RP was only partially similar to the structure of the analogous determinants of isolated porins. Most probably, “linear” antigen determinants corresponding to the separate regions of amino-acid sequence, were common for both proteins, and differences were observed at the level of “conformational” determinants, formed in the process of assembling of tertiary and quaternary protein structures.

It is established that, in spite of the partial correspondence between antigenic structure of RP and of porins solubilized from OM, the application of the recombinant analog of immunodominant protein as the diagnostic antigen, increased the sensitivity of the ELISA test-system for the verification of pseudotuberculosis various forms. It was shown that with the aid of RP the specific antibodies were revealed 1,3 times more effectively in the sera of patients both with intestinal and secondary forms of pseudotuberculosis.

METABOLITES FROM THE MARINE ACTINOBACTERIUM STREPTOMYCES SP. KMM 7210

Sobolevskaya , M.P., Leschenko, E.V., Kuznetsova, T.A., Denisenko, V.A., Shevchenko, L.S.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: kuztata@piboc.dvo.ru

4-Hydroxy-benzeneethanol (tyrosol) (**1**), together with 1-phenyl-ethanone (**2**), were isolated from the actinobacterium *Streptomyces* sp. KMM 7210. The chemical structures of the compounds **1** and **2** were established by spectroscopic methods and the mass spectrometric data.



1 **2**

This study was financially supported by the Russian Foundation for Basic Research (Projects: № 06-04-48578 and № 05-04-48211) and the Federal Agency for Science of the Russian Federation (Programs “Scientific schools” and “Collection of Marine Microorganisms (CMM)” and by grant of FEB RAS № 06-III-B-05-130.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE RECOMBINANT CRYPTIC PORIN FROM YERSINIA PSEUDOTUBERCULOSIS

Solovjeva, T.F., Khomenko, V.A., Likhatskaya, G.N., Isaeva, M.P., Stenkova, A.M., Kim, N.Yu.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
E-mail: soltaf@mail.ru

Scrunity of the Y. pseudotuberculosis genome, using a porin specific gene probe, revealed the presence of cryptic gene very similar to that of E. coli encoding the minor porin Omp N. This gene was cloned, and its nucleotide sequence was determined. The deduced amino acid sequence contained a 339-residue mature protein, with a calculated mass of 37584 Da. The multiple alignment indicated substantial sequence homology of OmpN to the known nonspecific Y. pseudotuberculosis porins. Its sequence is 58%, 51 % and 56% identical to those of OmpF, OmpC and PhoE respectively.

A highly conserved sequence motif, PEFGGD, and five charged residues(R 35, R 74, D 108, E 109, and R 125) which form a strong transversal electrostatic field in the channel interior are present at the same positions as in the superfamily of nonspecific porins. Comparison of C-terminal sequences Omp N and other Y. pseudotuberculosis porins revealed a Gln to Arg substitution at position 2 from the C-terminus in Omp N. The presence of a positively charged residue (Arg or Lys) at the penultimate position is not the case in enterobacterial outer membrane proteins (OMPs). This residue appears to be involved in the species specificity of OMPs recognition with assembly machinery.

The protein OmpN was expressed in a porin-deficient mutant E. coli strain. It was isolated from cells according to the procedure reported by Rosenbusch and was purified by size-exclusion chromatography. The recombinant OmpN porin migrates in SDS-PAGE without heat denaturation as 90 – 100 kDa protein, which collapses into about 38 - kDa monomer on heating in SDS. This result suggests that it exist as an oligomer. However the admixture of monomer is observed in the unboiled sample of recombinant OmpN on SDS-PAGE, pointing to a diminished stability of the oligomeric state and thus of the whole porin. Unlike nonspecific Yersinia porins, the recombinant OmpN oligomers were found to be destabilized by long exposure to SDS. On standing in 1% SDS they form the aggregates which are partly soluble on boiling in conventional SDS-PAGE sample buffer. This result supports the conclusion that the recombinant OmpN oligomers are insufficiently stable.

The far UV CD spectrum of the Omp N is consistent with those obtained for other proteins that retain characteristic structural features of porins. The minimum observed at 218 nm suggests that this porin is comprised predominantly of β-pleated sheet structure. The three-dimensional model of the Omp N was obtained using the homology modeling approach and 3-D structure of PhoE porin from *E. coli* as template. Based on this structure model, Omp N monomer has a β-barrel structure with 16 antiparallel transmembrane β-strands, eight short periplasmic turns and eight external loops. The loop L2 protrudes from the barrel. The longest loop L3 ($Gly^{95}\rightarrow Ala^{124}$) folds into barrel. This loops are characteristic of many porins as loops responsible for the size-selectivity of the channel and the structural integrity of porin. The secondary structure calculated for the model is in a good agreement with the experimental data obtained from CD spectrum.

The porin activity was determined in reconstitution experiments using artificial planar lipid bilayer membranes. The average value of the Omp N channel conductance of 180 ± 20 pS is the same as for the Y. pseudotuberculosis OmpC trimer porin. The distributions of the conductance steps were essentially unimodal with some smaller peakes at higher conductance values that presumably report simultaneous insertions of some porin molecules. However within a few hours after the onset of the BLM experiment the conductance curve of the Omp N became noisy. Furthermore, the similar result was obtained for the OmpN after 3 hr of porin exposure in diluted solution (10 ng/ml). The noise is most likely caused by structural fluctuations of the inserted pores. Such phenomenon has been described for E. coli mutant porins which have labile trimer structure.

Collectively, the above data demonstrate that wild-type OmpN trimer expressed in E. coli is prone to degradation and aggregation quite possible because either a special structure feature or incorrect assembly in the heterologous host.

OmpF GENE, AS PUTATIVE PHYLOGENETIC MARKER OF *YERSINIA SPECIES*

Stenkova, A.M., Isaeva, M.P., Belaykova, E.P., Guzev, K.V., Shubin, F.N., Rasskazov, V.A.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: stenkova@gmail.ru

Yersinia pestis, *Y. pseudotuberculosis*, and *Y. enterocolitica* have clearly been shown to cause human disease, while characterization of the remaining nine of Yersinia species often referred as "Yersinia enterocolitica-like" strains has been more limited. Recently, however, these species thought to be nonpathogenic to humans have been found to possess novel virulence mechanisms, and some of them have been associated with human disease. Identification of these species is a problem for clinical microbiology laboratories because the bacteriological and biochemical methods are often laborious and give ambiguous results. Molecular-genetic methods are the most advance and promising. It is common knowledge that for phylogenetic analyses of bacteria widely used such as 16S rDNA and gyrB genes.

In this study we analyzed possibilities of used ompF gene, as phylogenetic marker of the Yersinia species. This gene encode OmpF porin – the major, canal-former protein of the outer membrane of gram-negative bacteria. Primary structure of the OmpF porins of Enterobacteriaceae contain high-homology regions (transmembrane β-strends) and high-heterology regions (internal α-helix). We design sequencing primers for the Yersinia ompF, 16S and GyrB genes. PCR amplification and direct sequencing of these genes from 12 Yersinia strains were performed. Analysis and multiple alignment of nucleotide sequences, construction of the phylogenetic trees were carried on MEGA4 program software. NCBI database was searched using the BLAST programs. Comparative analysis of the 16S rDNA sequences showed high homogeneity (98-100%) within genus Yersinia. It is impossible to determine separate species by 16S rDNA. Analysis of the gyrB partial sequences showed less homogeneity (88-100%), but *Y. pseudotuberculosis* and *Y. pestis* form homogeneous group with 100% similarity, which affirm its close evolution and relationship. The most heterogeneous species were *Y. enterocolitica* and *Y. frederiksenii*. Fist of them formed 3 separate groups, second formed 2 groups with diversity more 10%. Analyses of ompF gene sequences showed ideal division, which allowed separate so homogeneous species as *Y. pseudotuberculosis* and *Y. pestis*. Also in other species the subdivision was observed and closely correlated with data obtained from the gyrB analyses. Thus, it was shown that the ompF gene allow to difference Yersinia species, and can be potential phylogenetic marker not only Yersinia but also other Enterobacteriaceae.

NEW MARINE NATURAL PRODUCTS: STRUCTURES AND ACTIVITIES

Stonik, V.A.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: stonik@piboc.dvo.ru

The first chemical studies on natural products took place in Russia during the second half of the 19th – early 20th century (the structure of α-pinene and so-called camphene rearrangements, established by E. Wagner and the first developed chromatographic separation by M. Tsvet). In the former Soviet Union time, well known scientific schools under the leadership of Professors A. Orekhov (alkaloids), M. Shemyakin (antibiotics), L. Bergelson (lipids), I. Torgov (steroids), N. Kochetkov (carbohydrates), and others were successfully operating, mainly in Moscow. Later, the Pacific Instutute of Bioorganic Chemistry (PIBOC) became more marked center of the studies on natural products in Russia.

Scientists from PIBOC actively participated in studies on marine natural products. For instance, during recent three years more than hundred new natural products from marine invertebrates and algae have been isolated at the Institute. A big series of new triterpene glycosides, including so-called non-holostane ones were isolated from sea cucumbers *Synapta maculata*, Far-eastern *Cucumaria ochotensis*, New Zealand and South Australian *Australostichopus mollis* and their physiological activities were investigated (S. Avilov, V. Kalinin, S. Fedorov et al.). Extremely potent immunostimulatory properties of these and related natural products were discovered and used to select pharmaceutical leads for further preclinical and clinical trials. Unexpected so-called two-headed sphingolipid sponge metabolites were found and studied, for example rhizochalines C and D, a rare threo-sphingolipid (T. Makarieva et al.). Many dozens new steroid metabolites from echinoderms and sponges (A. Kicha, E. Levina, T. Makarieva et al.) were described. The first marine dioxin derivatives and numerous new alkaloids, for instance ophiuroidin (N. Utkina), were discovered and have made a substantial contribution to the chemistry of marine natural products. Several natural products with rare and previously unknown skeleton system were found, exemplified by a cyclopropane-containing polyhydroxysteroid phrygiasterol from the Pacific starfish *Hippasteria phrygiana* and an unusual spongian diterpenoid from the tropical sponge *Heterofibria* sp.

Some isolated compounds demonstrate interesting biological activities, including cancer preventive properties (terpenoids from ascidians), the stimulation of neuron differentiation and the growth of neurites (starfish steroids), potent antioxidant properties (alkaloids from brittle stars), spermostatic effects (sponge oligoglycosides), antifungal action (bipolar sphingolipids from sponges).

A series of new marine natural products were synthesized, including 3-demethylubiquinone Q2 from the Far-eastern tunicate *Aplydium glabrum*.

Molecular mechanisms of biological action were established in some cases. The studied sea cucumber triterpene gycosides induced apoptosis of tumor cells influencing on several known proapoptotic pathways. Dactylone from the mollusk *Aplysia dactylomela* was effective in non-toxic doses as a cancer preventive agent, which exerted its actions through inhibition of cyclin D3 and Cdk4 expression and retinoblastoma tumor suppressor protein phosphorylation. The inhibition of these cell cycle components was followed by arrest of G(1)-S transition with subsequent p-53-indepenent apoptosis.

As a result of the recent works of Russian scientists from PIBOC, many new natural products were obtained. It contributed into the knowledge concerning the biochemical diversity in marine biota, providing new data on structures and properties of natural products, as well as created a basis for development of drugs and food supplements, based on natural products and their derivatives and/or analogs.

INVESTIGATION OF INFLUENCE OF RADIANTHUS PORE-FORMING TOXINS ON CELL CULTURES

[1]Tabakmakher, V.M., [2]Monastyrnaya, M.M., [1]Dyshlovoy,S.A., [2]Fedorov, S.N

[1]Far-Eastern National University, Vladivostok.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: tabval@yandex.ru

Pore-forming toxins of sea anemones (actinoporins) are sphingomyelin-inhibited cytolytic polypeptides (cytolysins). They have a great variety of biological activities because they can be potentially used to design new medicines. Actinoporins are also used as instruments for studying molecular organization and mechanisms of biological membranes functioning [1].

Actinoporins RTX-SII and RTX-A were isolated from sea anemone *Radianthus macrodactylus* previously with the methods of water extraction and liquid chromatography [2, 3]. According to MALDI-TOF MS data RTX-SII's molecular mass is 19.28 kDa. RTX-A's mass was estimated according to amino acid composition as 17.249 kDa. The values of hemolytic activity of RTX-A and RTX-S II are 3.5×10^4 and 3.6×10^4 hemolytic units per mg of peptide, respectively. According to the current hypothesis two actinoporin's fragments, phosphocholine binding site and N-terminal fragment participate in pore formation [4].

The study of Radianthus actinoporin's action on fertilized urchin eggs showed a maturation division of eggs in spite of absence of sphingomyelin in their membranes (data not published).

In this work we studied cytotoxic action of sum actinoporins RTX-SII and RTX-A (equimolar mixture) against HeLa (cervical carcinoma), JB6 Cl41 JNK and JB6 Cl41 p38 (mouse epithelial cells) using MTS-method [5]. The mixture showed high activity: IC_{50} = 2.45 μM against HeLa cells, IC_{50} <12.5 nM against JB6 Cl41 JNK and JB6 Cl41 p38 cells. Further investigation will be applied to find out the mechanisms of Radianthus actinoporins anti-cancer activity. Obtained results and earlier dates testify the participation of another functionally important molecular fragment (e.g., RGD-motive, which is known to interact with cell membrane integrins) in actinoporins' action mechanism.

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CLONING, SEQUENCING, AND EXPRESSION OF ACTINOPORIN FROM THE SEA ANEMONE, *RADIANTHUS MACRODACTYLUS*

Tkacheva E.S., Eskov A.A., Leychenko E.V., Isaeva M.P., Guzev K.V., Monastyrnaya M.M., Kozlovskaya E.P.

Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: leychenko@piboc.dvo.ru

Actinoporins are unique by they structure and properties class of biological active compounds of the sea anemones. The investigations showed that actinoporins reveal a wide spectrum of biological activity such as cytolytic, cytotoxic, cardiotropic, dermatonecrotic, anticancer [1-4]. Today defined attention turn to research of cytotoxic action of actinoporins in vivo and in vitro on the target cells and target organs (including different cell cultures), and also profound attention turn to getting the immunoconjugates of actinoporins with different ligands, that will help to create the prerequisites for making structural constructions ("framework") of immunotoxins (the potential pharmaceutical agents).

Recently our attention is directed to structure and properties study of actinoporins from the tropical sea anemone *Radianthus macrodactylus*. In this work a full-length actinoporin cDNA was obtained from the sea anemone *R. macrodactylus* cDNA library by combination of methods involving RT-PCR, 3'- and 5'-RACE. The obtained nucleotide sequence of cDNA contains an open reading frame of 627 base pairs, which encodes a protein of 209 amino acid residues. The nascent protein contains a signal peptide, a propart and a mature protein (19, 15 and 175 amino acid residues, respectively). The predicted molecular mass of the mature protein, actinoporin RTX-A, is 19307.1 Da, and the isoelectric point - 9.1. Amino acid sequence of RTX-A contains a Arg-Gly-Asp (RGD) motif, potential cell attachment, and possesses high homology (86-90%) with actinoporin sequences from sea anemones belonging to the same family Stichodactylidae. It was established that the β-structure predominates at the secondary structure of RTX-A by the predicted methods. The characteristic amphiphilic alpha-helix structure was also found in the N-terminal region of the actinoporin.

To obtain of the actinoporin recombinant form (rRTX-A) the construction of gene fragment encoding mature actinoporin with expression vector pET41a was designed and cloned. The conditions chosen and functional expression was curried out. The precursor of rRTX-A was obtained as GST-fusion protein containing His-tag on N-end of actinoporin. It was isolated both from cell lysate and LB medium by affinity chromatography. rRTX-A was isolated by reverse-phase HPLC after treatment of the precursor by enterokinase. At present the properties of recombinant protein are studying.

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TERPENOIDS OF SIBERIA FLORA IN WORKING OUT OF THERAPEUTICALLY PERSPECTIVE AGENTS

Tolstikov G. A.
N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, SB RAS, 630090 Novosibirsk, Academician Lavrentiev ave., 9, 630090 Novosibirsk, Russia
fax: (383)330-49-80, e-mail: gtolstik@nioch.nsc.ru

The results of program on working out of therapeutically perspective agents on the basis of synthetically transformations of sescvi-, di- and triterpenoids are presented. The named terpenoids are produced with the widely distributed forest tree and landscape Siberian plants and obtained by technological methods.

This program includes the follow works.

1. δ-Cadinol in the synthesis of eleuthesides and theirs analogues.
2. Synthesis of cytostatics of cembranoid type on the base of isocembrol.
3. Transformations of lambertianic acid as a base of producing of noothropic, psychothropic and cytotoxic agents.
4. Synthesis of new higher active antiviral agents and correctors of toxic effects from betuline.
5. The glycyrrhizic acid derivatives are new groups of antivirus (anti-HIV) and immunothropic agents.

NEW DATA ON THE STRUCTURAL DIVERSITY OF RED ALGAL POLYSACCHARIDES

Usov, A.I.
N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninskii Prospect 47, Moscow 119991, Russia,
e-mail: usov@ioc.ac.ru

The division Rhodophyta (red algae) contains about 4.000 species. They have a unique set of photosynthetic pigments and metabolic pathways leading to unique polysaccharide composition of the biomass. It is well known that red algae contain sulfated galactans which have never been found in other natural sources. Their molecules have a linear backbone built up of alternating 3-linked β-galactose and 4-linked α-galactose residues. The 3-linked galactose always belong to the D-series, whereas 4-linked galactose may be L or D, and this is the difference between agars and carrageenans. In addition, 4-linked residues may be partially or completely converted into 3,6-anhydro derivatives. As the result, there are four basic disaccharide repeating units, two of them being characteristic for polysaccharides of the agar group, and two others – for carrageenans. Structural diversity in each group results from different content of basic repeating disaccharides, their different distribution along the backbone, and various substitution of hydroxyls, which are often methylated in agars and sulfated in carrageenans. Several new structural features were found recently giving new insight on the structural diversity of red algal polysaccharides:

2. Highly substituted comb-like agarans named “corallinans” were isolated from representatives of the family Corallinaceae.
3. Highly methylated and pyruvylated polysaccharides were found in carrageenans.
4. The presence of both agar and carrageenan structures was detected in many complex galactans, thus confirming the absence of a border between agarophytes and carrageenanophytes.
5. Deviation from the strict alternation of 1→3 and 1→4 linkages in the galactan backbones was described several times, but this fact needs additional confirmation.
6. Polysaccharides of different structural types were found in several species, such as alginic acids in Corallinaceae, sulfated (1→3)-α-D-mannans in Nemaliales and (1→4)-β-D-mannans in Ceramiales, as well as neutral (1→3,1→4)-β-D-xylans in Palmariales.
7. Polysaccharide composition of several unicellular red algae, as well as of fresh-water species differs considerably from that of marine macrophytes described above.

The data on polysaccharide composition are important to predict the potential practical use of red seaweeds and may be helpful in elucidation of detailed taxonomic position of the species.

AROMATIC METABOLITES FROM MARINE SPONGES AND ECHINODERMS

Utkina, N.K.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: utkinan@mail.ru

Phenolic and heteroaromatic compounds are a large group of secondary metabolites having a wide range of biological activities. Majority of these metabolites has been isolated from terrestrial plants. Work on studying marine natural products has been started just 50 years ago and has demonstrated that owing to physical and chemical conditions in the marine environment, almost every class of marine organism exhibits a variety of molecules with unique structural features not found in terrestrial natural products.

We have investigated aromatic metabolites from large-scale representatives of marine invertebrates: echinoderms and marine sponges.

It was shown that naphthaquinonoid pigments isolated from sea urchins have highly hydroxylated patterns in contrast to plant naphthaquinones. These metabolites exhibit high antioxidant activity. Sulfated hydroxyanthraquinones have been found in starfishes of *Echinasteridae* family. The first indoloquinazoline alkaloid, ophiuroidine, having an indolo[2,1-*b*]quinazoline-6,12-dione skeleton, was isolated from the ophiuroid *Ophiocoma riisei*. Ophiuroidine is the trihydroxylated derivative of tryptanthrine, the active component of indigo plants.

Chemical diversity of phenolic metabolites and aromatic alkaloids is presented in marine sponges. One of the distinctive features of marine natural products as well as aromatic compounds is the presence of halogen in their structures.

A number of antimicrobial and cytotoxic polybrominated diphenyl ethers and polybrominated dibenzo-*p*-dioxins have been isolated from a small group of tropical marine sponges, namely, *Dysidea* species. Bromine content runs to 70% in some of these compounds.

A series of alkaloids, bearing a brominated pyrrol cycle, has been isolated from marine sponges of the families *Agelasidae* and *Axinellidae.*

Aromatic fragments are occurred in products of mixed biogenesis in marine sponges of the order Dictyoceratida. These compounds incorporate both sesquiterpene and hydroquinone or quinone moieties. Marine sesquiterpene phenols/quinones can differ from similar plant metabolites by the presence of a nitrogen-containing functionality or a halogen atom in a benzenoid part of a molecule. Sponge sesquiterpene quinones exhibit antioxidant, cytotoxic and hemolytic activities.

Singularity of marine derived aromatic compounds is not only limited to functionalities, but also concerns their carbon skeletons. A series of unusual cytotocsic aromatic alkaloids has been isolated from marine sponges, aaptamines (1,6-naphthyridins), zyzzyanones (a novel class of dipyrroloquinones), makaluvamines (pyrroloquinolines). Unusual skeletons of these compounds have not been found in plant alkaloids.

In conclusion, aromatic metabolites from echinoderms and marine sponges have displayed such structural features as high hydroxylation, halogenation, the presence of nitrogen and sulfur-containing functionalities, and unique cyclic skeletons.

Studied aromatic marine natural products possess antimicrobial, cytotocsic, hemolytic, antioxidant and UV-protective properties, and probably play a defensive role against infection, predators, and harmful environmental conditions.

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BIOLOGICALLY ACTIVE SUBSTANCES OF MARINE MACROPHYTES

Vaskovsky V.E. and Belous O.S.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
e-mail: vev35@mail.ru

Algae as well as bacteria and sponges are the most rich groups among marine organisms in different chemical compounds which have various biological activities. Marine algae are very heterogenic taxonomically. Usually they are subdivided into 10 groups including both micro and macroorganisms: Cyanophyta, Pyrrophyta, Chrysophyta, Bacillariophyta, Xanthophyta, Phaeophyta (brown algae), Rhhyta (red algae), Eglenophyta, Chlorophyta (green algae) and Charophyta. From the ancient times two groups of macrophytic algae, red and brown. And some less green algae were popular marine products in many terms. In costal regions of many countries they were used as food, for animal feeding and agricultural fertilizers. Some later they were used as industrial row materials to isolate iodine and mineral preparations. About last one hundred years very valuable polysaccharides are produced from brown and red algae. Some of these products are biologically active substances.

In the communication a short review of the investigation of different biologically active substances of algae are given. It is a topical problem. In period 2000 – middle of 2008 it published more papers than in 1991-1999. The input of different country in the publications changed considerably. In previous period the first place among producers of the information was occupied by Japan. USA was the next, other places in the first dozen were occupied European country and USSR. In the second period USA became the first, PRC and South Korea are in third and fourth places? Russia together with former republics of USSR (Ukraine, Georgia) is not in the first dozen.

Information on biological activity of algal polysaccharides, lipids terpenoids, alkaloids, phenolyc compounds were demonstrated. The next main kinds of biological activities were found: antibacterial, antifungal. antiviral, antioxidant, antifouling, anticancer, anticoagulant, hypolipidemic. Anti thrombosic, radio protective.

Recently a rather detailed review on practically important BAS of marine macrophytes containing about 200 references was published [1]. Therefore we pay the main attention to publications of 2004-2008 in our communication.

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Some data on feeding trends of common Mytilidae (Bivalvia) species from the Sea of Japan

[1]Vekhova, E.E., [2]Kusaykin M.I.
[1]*A.V. Zhirmunsky Institute of Marine Biology FEB RAS*, evekhova@gmail.com
[2] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*

The growth, successfully development and normal vital activity of the mussels greatly depend on food supplies. It is widely known that bivalve mollusks of the family Mytilidae are the filter-feeders. They filter bacteria, plankton, and detritus from the water column above mussels. There are insufficient data on the portion of each of these feeding components in the mussel's diet in the literature up to date. In this connection, the aim of our study was to examine the activity of the digestive enzymes, like O-glycosyde hydrolases, which catalyze splitting of the primary substance (1→3-β-D-glucan) containing in phytoplankton in common mussel species of coastal communities of the Sea of Japan, − Grayan's mussel *Crenomytilus grayanus* (Dunker, 1853), the Korean mussel *Mytilus coruscus* Gould, 1861, northern horse mussel *Modiolus modiolus* (Linnaeus, 1758), and Bay mussel *Mytilus trossulus* Gould, 1850. As a rule these mussel species have the similar features of lifestyle, the way of feed and other biological characteristics.

Mollusks were collected from February to November during 2006, 2007 from the mussel beds from the different parts of Sea of Japan (Vostok Bay, Amur Bay, Ussuriisk Bay) at depth of 0.5–6 m using scuba-diving equipment. The tissues of digestive system of 3–5 living speciments of each mussel species were analyzed three times. Activity of the digestive enzymes was determined by the method of Nelson (Nelson, 1944). Each samples were carefully disintegrated and extracted with 0.05 M succinate buffer (pH 5.2) in ratio 1:3. Extraction was centrifugated during 15 min at the rate of 6000 overturn/min, at the temperature +4°C; 1 ml of supernatant was exposed to gel-filtration on a column G-25 for removal of the low-molecular dirts (Sova et al., 1970). The digestive activity of the mussels was estimated on the level of enzymatic activity 1→3-β-D-glucanases (laminarinases), which was the most high in comparison with remainder enzymes O-glycosyde hydrolases.

The conducted comparative investigation allow to confirm the following:

- ✓ The activity level of the enzymes O-glycosyde hydrolases in *C. grayanus*, *M. coruscus, M. modiolus*, and *M. trossulus* decreases in the range: 1→3-β-D-glucanases (laminarinases) – 1→6-β-D-glucanases (pustulanases) – glucosidases – manosidases –galactosidases. All examined mussel species have sufficiently high the activity level of 1→3-β-D-glucanases.
- ✓ The investigated mussel species with the equal linear dimensions of the shell have different specific activity of 1→3-β-D-glucanases. In the mussels measuring 85 mm in size, for example, in *M. coruscus*, the mean specific activity of 1→3-β-D-glucanases is higher in 2.5 times than in *C. grayanus*, and 2 times than that in *M. modiolus.* The most high level of specific activity of 1→3-β-D-glucanases irrespectively of the shell length has *M. trossulus*. In the ontogenesis of *C. grayanus*, *M. coruscus, M. modiolus*, and *M. trossulus* the activity level of 1→3-β-D-glucanases is variated. Thereby it argues that during the ontogenesis *C. grayanus*, *M. coruscus, M. modiolus*, and *M. trossulus* feed on the phytoplankton in a different amount.
- ✓ The observed differences in the activity level of 1→3-β-D-glucanases apparently relate with the spatial distribution of the mussels in marine coastal zones. Taking there only on the open coastal rocky grounds with heavy hydrodynamical conditions, the mussels *M. trossulus and M. coruscus* feed on the phytoplankton most actively than that *C. grayanus* and *M. modiolus*, which occupy deeper sites of the bottom of those coastal areas that are protected from waves. Nevertheless a phytoplankton makes an essential component of food *Crenomytilus grayanus*, *Modiolus modiolus*, *Mytilus coruscus* and *Mytilus trossulus*, however each mussel species consumes it in different amount.

ISOFLAVONOIDS FROM *MAACKIA AMURENSIS* CELL CULTURE

M.V. Veselova[1], S.A. Fedoreyev[1], V.P. Bulgakov[2], O.E. Krivoschekova[1]

[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*

[2] *Institute of Biology and Soil Science, Far East Branch of Russian Academy of Sciences, Vladivostok, Russia*

e-mail: veselova@piboc.dvo.ru

Isoflavonoids, an interesting and restricted group of secondary metabolites of legumes, exhibit estrogenic, antiangiogenic and anticancer activities, and are now popular as dietary supplements [1-3]. *Maackia amurensis* Rupr. et Maxim. is the only woody plant representative of Leguminosae family in the flora of the Russian Far East. This species is a relict tree of the tertiary flora [4]. The polyphenolic complex from the heartwood (PHW) of *M. amurensis*, named maksar preparation, has been registered in Russian Federation as a hepatoprotective drug in 2004. Both polyphenolyc complexes extracted from the heartwood (PHW) and the callus cultures (PHC) of *M. amurensis* decreased the acute toxicity of tetratrachloromethane on liver with similar efficiency, showing a prominent favorable effect on animal survival. This effect was due to the suppression of the dystrophy and necrosis of hepatocytes, the normalization of aminotransferase and γ-glutamyltransferase activities in the blood, and the stimulation of bilirubin conjugation [5-7]. The HPLC and NMR analyses of PHW yielded monomeric and dimeric stilbenes, isoflavones, pterocarpans [4].

In the present investigation, we provided the detailed chemical analysis of the polyphenolic complex isolated from the most productive A-18 callus line of *M. amurensis*. We initiated the research of plant cell cultures possessing increased ability to synthesize these polyphenolic compounds. Cell cultures of *M. amurensis* were established during the investigation. On data HPLC and NMR analysis this culture produces 20 isoflavonoids, namely isoflavones: genistein, daidzein, formononetin, calycosin, derrone, pseudobaptigenin; their glycosilated conjugates: genistin, 6"-*O*-malonylgenistin, ononin, 6"-*O*-malonylononin, daidzin, 3'-metoxydaidzin, 4'-*O*-β-D-glucopyranosyldaidzin, 4'-*O*-β-D-glucopyranosylgenistin, 7-*O*-β-D-glucopyranosylcalycosin; pterocarpans: maackiain and medicarpin; their glycosilated conjugates: 6"-*O*-malonyl-3-*O*-β-D-glucopyranosylmaackiain, 6"-*O*-malonyl-3-*O*-β-D-glucopyranosylmedicarpin; and a new pterocarpan glucoside 6"-*O*-malonyl-3-*O*-β-D-glucopyranosyl-6,6a-dehydromaackiain. The A-18 calli, which have been established in 2001, were analyzed for two years (2006-2007) by HPLC. During this time, the calli produced in total 1.437±0.178% DW of isoflavonoids. The dynamics of polyphenol accumulation during a subculture was characterized by continuously increasing levels of polyphenols up to 45-50 days (stationary phase of the growth); after that their levels declined. Interestingly, despite *M. amurensis* plants produced large amounts of stilbenes, we could not detect any substantial quantities of these substances in our callus cultures. On the other hand, we had not found in PHW, isolated from the heartwood of the *M. amurensis*, glycosilated conjugates of the isoflavones and pterocarpans.

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STUDY ON THE POLYSACCHARIDE AND OLIGOSACCHARIDE FROM *AURICULARIA AURICULA*

Li Wei, Song Yue, Zhang Jinling, Jin Qiao, Tong Chang-qing
Dalian Fisheries University, 52 Heishijiao, Dalian 116023, China

e-mail: aisingioro@mail.ru

Auricularia auricula have long been used as a food and a drug in China. Its fruit body is a black-brown mushroom with high contents of polysaccharides and protein. In recent years, *A. auricula* polysaccharide (AAP) was found to have many biological activities. The polysaccharide in the fruit bodies was extracted with hot distilled water. The molecular mass was further determined by MALDI-TOF mass spectrometry. The spectrum of AAP contained 3 peaks at *m/z* 2.1, 9.5 and 18.8 kDa. The AAP havs better antihypertensive effects on renovascular hypertensive rats. AAP were hydrolyzed by 2 M CF_3COOH to obtain a mixture of oligosaccharides which were then separated by DE 52 and Sephadex-G 25. The pectin was isolated from *A. auricula* by acid heating method. The molecular mass of pectin by MALDI-TOF was 18.9 kDa. The biological activities of oligosaccharides of *A. auricula* are under progress in our laboratory.

UV-PROTECTIVE SUBSTANCES IN THE TISSUE OF RED AND BROWN SEAWEEDS FROM THE JAPANESE SEA, RUSSIA

Yakovleva, I.M., Nabivailo, J.V.
A.V. Zhirmunsky Institute of Marine Biology Far Eastern Branch of the Russian Academy of Sciences, Palchevskogo str. 17, Vladivostok 690041, Russia, Fax: 7 4232 310900

e-mail: yakovleva72@mail.ru

Experimental evidence of ultraviolet (UV)-screening properties and antioxidant potential for phenolic compounds in the tissue of brown seaweeds and for mycosporine-like amino acids (MAAs) in the tissue of both red and brown representatives is reported. The results show that total contents of phlorotannins and MAAs were positively and highly significantly correlated with the diurnal and seasonal cycles of ultraviolet (UV) radiation. The diurnal and seasonal increases in total MAA concentrations were due to an increase in the concentration of imino-MAA species. Seasonal nitrate concentrations in seawater were also positively correlated with MAA level in all species investigated at the time of the sampling. This confirms UV-screening function of imino-MAA species and phenolic compounds, and suggests a negative effect of nitric deficiency on MAA biosynthesis. It is experimentally established that oxo-carbonyl-MAA, mycosporine-glycine (Myc-Gly), and phlorotannins decreased reactive oxygen species toxicity before an activation of the antioxidant enzymatic systems and functioned in the algal tissue as first aid to oxidative stress. A high UVR tolerance of intertidal algal species observed at midday hours and in March and August (the periods of the highest annual solar insolation) was associated with the great abundance of phenolic compounds and Myc-Gly. This suggests that predominance, in the seaweed tissue, of MAA species and/or phenolic compounds with antioxidant ability may render seaweeds more tolerant to oxidative damage induced by irradiance, temperature and other abiotic factors in the intertidal zone.

THE MARINE POLYSACCHARIDES AS ENDOTOXIN - NEUTRALIZING AGENTS

[1]Yermak, I.M., [1]Davidova, V.N., [1]Barabanova, A. O., [1]Gorbach, V.I., [2]Khasina E.I., [1]Solov'eva, T. F.

[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*
e-mail: iren@piboc.dvo.ru

The lipopolysaccharides (LPS) of gram-negative bacteria or endotoxin exhibits a variety of biological activities in mammal that may be beneficial at low concentrations but pathophysiological at higher levels due to an overproduction of cytokine in immune cells such as interleukins (IL) and tumor necrosis factor alpha (TNF-α). The effect of marine polysaccharide – chitosan and carraggenans - upon some biological properties of LPS from *Yersinia pseudotuberculosis, E. coli* and *Proteus vulgarius* was study.

Chitosans (β-1,4-glucosaminoglycan, 120 kD and 30 kDa) were obtained by alkaline treatment of crab shell and by further depolymerization. Carrageenans - sulfated galactans of different structures were isolated from red algae family of Gigartinaceae and Tichocarpaceae collected from the Russian Pacific coast.

An addition of chitosan (Ch) to LPS solutions was found to result in formation of the stable LPS-Ch complexes of different stoichiometry in which negative charge of LPS was neutralized (LPS from *E.coli*) or overcompensated (*Y. pseudotuberculosis* and *P. vulgaris).* The interaction is a complicated process and depends on time and reaction temperature, as well as on the molecular weight of chitosan and structure of LPS (surface charge of LPS particles, lengths of O-specific polysaccharide, and also containing of fatty in lipid A).The LPS from *E. coli* possess higher affinity to chitosan in comparison with two others samples of endotoxins. The conversion of the ultra structure and sizes of LPS was observed to result action of carrageenans.

Chitosan and carrageenans was shown to modulate significantly the biological activity of LPS. LPS with chitosans and carrageenans was found to posses much lower toxicity in a comparison with the parent LPS. Chitosan and carrageenans was shown to maintain an ability of LPS to induce of IL-8 and TNF. As know LPS induce the production of cytokines in monocytes/macrophages through TLR-4 receptor. Chitosan-induced secretion of the pro-inflammatory cytokines in macrophages is not dependent on TLR4 and chitosan do not block the TLR4-receptor for LPS binding and LPS signal transduction in the cell. The activation of cells by carrageenan occurs through specific for LPS TLR-4 receptors and the ability of carrageen to induce cytokine production plays an important role in the modification of the toxicity of LPS.

The study showed that chitosan and carrageenans increase non-specific resistance to impact of LPS-induced endotoxinemia in mice. Polysaccharides were administered intragastrically during 5 days followed by intra peritoneal injection of LPS. Chitosan and carrageenan hampered the involution of thymus, hypertrophy of adrenal glands, the changes on level of thyroid hormones an corticosterone in serum, the activation of glycogenolysis, glycolysis and peroxidation of lipids in liver.

The capability of carrageenan to correct parameters of the hemostatic system, and also its influence on the homeostatic and immune system in the course of treatment of patients with food toxic infection of *Salmonnela* etiology was investigated. Carrageenans restored system of a hemostatic and parameters of immune system are more active, in comparison with control group. The obtained positive effect necessitates further research work in this direction.

ENDO-1,3-β-D-GLUCANASE FROM VIETNAMESE MUSSEL *PERNA VIRIDIS*

[1]Zakharenko, A.M., [1]Kusaykin, M.I., [2]Bui Minh Ly, [2]Pham Van Huyen,
[2]Hoang Nhu Khanh, [1]Sova, V.V., [1]Zvyagintseva, T.N.
[1] *Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia*
[2]*Nhatrang Institute of Technology Research and Application VAST, Nha Trang, Hung Vuong St. 02, Vietnam*
e-mail: Rarf@yandex.ru

(1→3)-β-D-Glucanases (laminarinases) belong to O-glycosylhydrolases, which are basic enzymes in carbohydrate metabolism. These enzymes are widespread among various organisms, from archaebacterium to eukaryotes. (1→3)-β-D-Glucanases are involved in various physiological processes. Particularly, they take part in digestion of bacterium cell walls. They participate in lysis of extracellular matrix glucans at the cell development of fungi. In higher plants (1→3)-β-D-glucanases cleave (1→3)-β-D-glucans at germination of seeds and act as inducible defense enzymes in protection plants from pathogenic fungi. (1→3)-β-D-Glucanases play important role in digestion and embryogenesis of invertebrates.

Marine invertebrates arouse especially interest. They are represented by a great number of taxons, that are at various steps of evolution and differ from each other in the way of a feeding and life habits. Marine invertebrates are rich and rather accessible sources of different O-glycosylhydrolases. The screening leaded earlier showed, that among marine invertebrates, living in various regions of the World ocean, the highest level of (1→3)-β-D-glucanase activity is observed in digestive glands of marine molluscs and crustacea.

The most important feature of (1→3)-β-D-glucanase of marine mollusks is that it possesses high transglycosylation activity, which is successfully used in enzyme synthesis of new compounds and transformation natural glucans for increasing of their biological activity.

The specific (1→3)-β-D-glucanase with molecular mass of 33 kDa was purified to homogeneity from the crystalline styles of Vietnamese commercial species mussel *P. viridis* by combination of different types of chromatography. The (1→3)-β-D-glucanase had narrow substrate specificity and hydrolyzed only the (1→3)-β-D-glucosidic bonds in the mixed (1→3);(1→6)- and (1→3);(1→4)-β-D-glucans down to glucose and glucooligosaccharides. This enzyme acted with retention of the anomeric configuration and catalyzed transglycosylation reaction. The K_m values of (1→3)-β-D-glucanase in the reaction with laminaran was 0.3 mg/ml. The enzyme displayed the maximal activity within the pH range 4.0-6.5 and temperature 45 ºC. Half inactivation time of enzyme at 45 °C was 180 min and at 50 ºC was 20 min. The enzyme was classified as glucan endo-(1→3)-β-D-glucosidase (EC 3.2.1.39). It is possible to use this homogeneus enzyme for establishment of the structure of some glucans and for enzymatic synthesis of new carbohydrate substances.

INHIBITION OF ADHESION OF *CORYNEBACTERIUM DIPHTHERIAE* TO HUMAN BUCCAL EPITELIUM BY GLYCOSIDE HYDROLASES OF MARINE HYDROBIONTES

[1]Zaporozhets, T.S., [1]Makarenkova, I.D., [2]Bakunina, I.Y., [2]Burtseva, Y.V., [2]Kusaykin, M.I., [2]Balabanova, L.A., [1]Besednova, N.N., [2]Zvyagintseva, T.N., [2]Rasskazov, V.A.

[1]Recearch Institute of Epidemiology and Microbiology, the Russian Academy of Medical Sciences, 1, Selskaya str., Vladivostok, 690087, Russia
e-mail: niiem_vl@mail.ru
[2] Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia

At present report a possibility of adhesion inhibition of *Corynebacterium diphtheriae* to human buccal epithelium by glycoside hydrolases of marine hydrobiontes was investigated.

For evaluation of possibility of bacterial adhesion inhibition α-galactosidase from marine bacterium *Pseudoalteromonas* sp. KMM 701 (*Bakunina I.Yu., et all,* 1998), total preparation of enzymes and β-1,3-glucanase from marine fungi *Chaetomium indicum* (*Burtseva Yu.V. et al.,* 2003), total preparation of enzymes and β-1,3-glucanase from marine mollusk *Littorina kurila* (*Pesentseva M.S. et al.,* 2008), and total preparation of enzymes from crystalline style of marine mollusk *Spisula sachalinensis* (*Sova V.V. et al.,* 1970) were used. The enzymes were added to test-tubes containing the cells of buccal epithelium and/or the bacteria of toxigenic strain *C. diphtheriae* № 1129*, v. gravis*. The suspension was incubated for 30 min at 37ºC, washed up thrice to remove the bacteria unattached to epithelocytes. Sample smears were visualized under a microscope. Number of the bacteria attached to epithelocytes was estimated. The mean of quantity of the bacteria attached to one epithelocyte was taken for analysis as mean adhesion index.

On present showing, all investigated enzymes were able to abort C. *diphtheriae* adherence to human buccal epithelocytes. Inhibition of adhesion was more marked in case of treatment of epithelocytes with high purified enzymes of marine hydrobiontes in comparison with total enzyme preparations. The significant inhibition of C. *diphtheriae* adhesion was observed when the enzymes were added to the epithelocytes with the attached microorganisms.

The results obtained showed that glycoside hydrolases of marine hydrobiontes degrades any carbohydrates expressed on cell surface of bacterium or human buccal epithelocytes, braking unique and specific of lectin-carbohydrate interaction and preventing the adhesion.

The perspective of subsequent study on possibility of application of marine hydrobionte enzymes as antiadhesines is discussed.

1. *Bakunina, I.Y., Sova V.V., Nedashkovskaya O.I., et al.* (1998) Biochemistry (Moscow), **63,** 1209-1215.
2. *Burtseva Yu.V., Verigina N.S., Sova V.V. et al.* (2003) Mar. Biothechnol, **5,** 349-359.
3. *Pesentseva M.S, Kusaykin M.I., Anastyuk S. et al.* (2008) Carbohydr. Res., in press.
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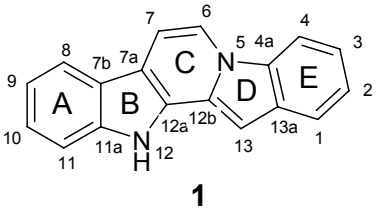
MODELLING, SYNTHESES AND BIOASSAYS OF SOME DERIVATIVES AND ANALOGS OF MARINE ALKALOID FASCAPLYSIN

Zhidkov M. E.
Pacific Institute of Bioorganic Chemistry Far-Eastern Branch of the Russian Academy of Sciences, 690022, Vladivostok, pr. 100-let Vladivostoku, 159, Russia
Far-Eastern National University
e-mail: mzhidkov@rambler.ru

The 12H-pyrido[1,2-a:3,4-b']diindole ring system (**1**) forms the framework of several marine alkaloids, such as fascaplysin (**2**). This natural product has a broad range of bioactivities including specific inhibition of Cdk 4 and DNA intercalation. This fact demonstrates the huge potential of fascaplysin derivatives for therapeutic assays and necessitates the elaboration of effective methods for their syntheses.



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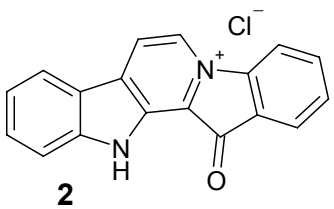
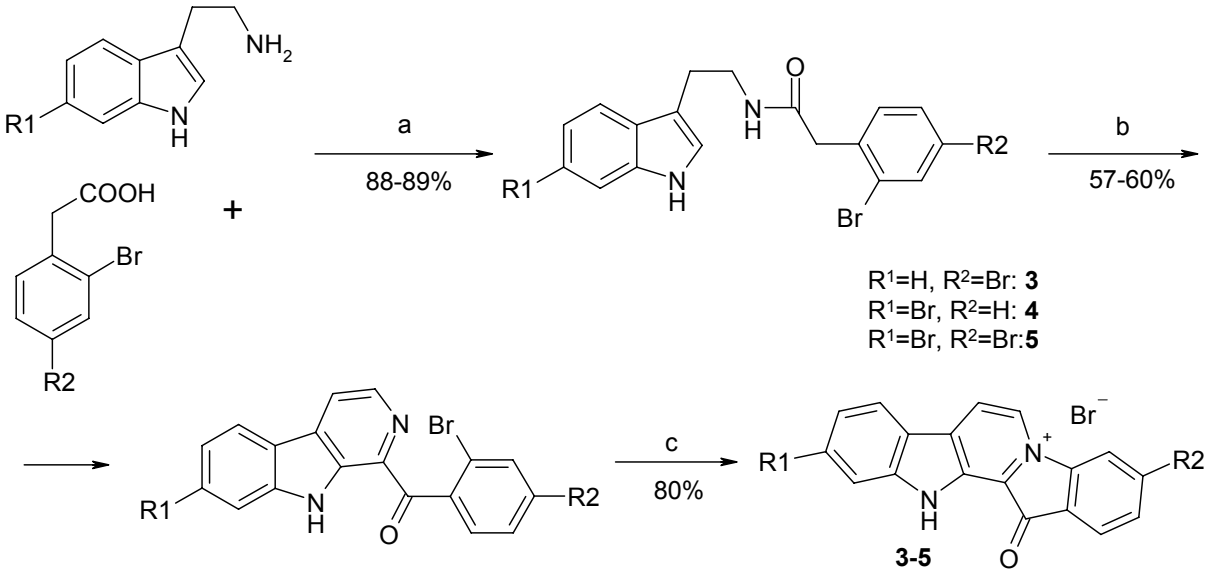


2

This project includes modelling and syntheses of some derivatives and synthetic analogs of fascaplysin via a simple approach involving ring system **2** formation, which we have elaborated for the synthesis of some natural bromosubstituted fascaplysin derivatives: 3-bromofascaplysin (**3**), 10-bromofascaplysin (**4**) and 3,10-dibromofascaplysin (**5**) (see Scheme).



a: DCC, CH_3CN, reflux, 30 min; b: $POCl_3$, CH_3CN, Ar, reflux, 40 min, then MnO_2, PhH, reflux, 3 h; c: 220^{0}C, 20 min.

These substances must have huge bioactivities. Their syntheses will open up fresh opportunities for detailed studies of the structure activity relationships among these potentially physiologically active compounds.