BIOLOGICAL ACTIVITY OF FEIGRISOLIDES A, B AND C FROM A MARINE ISOLATE OF THE ACTINOBACTERIUM *Streptomyces* sp. 6167.

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ABSTRACT

It was shown, that the macrolide metabolites from the marine derived actinobacterium *Streptomyces* sp. 6167, feigrisolides A, B and C are effectors of the 1→3-*β*-D-glucanase from *Spisula sacchalinensis* crystalline style. It was found that feigrisolide B induces apoptotic process in the Ehrlich carcinoma cells (IC_{50} =17.4 μg/ml), possess cytotoxic activity against the developing eggs of the sea urchin *Strongylocentrotus intermedius* and immobilizes sperm of the urchin in dose less than 1 μg/ml.

Keywords: *macrolides, feigrisolides, biological activity, marine actinobacterium Streptomyces sp.*

1. INTRODUCTION

Actinomycetes, including the common genus *Streptomyces*, isolated from the marine environment are a proven source of structurally diverse natural products, possessing broad-spectrum of biological activities [1-3]. They are of great interest as sources of lead compounds for the pharmaceutical industry, since a large number of naturally occurring bioactive substances are produced by the order Actinomycetales. Macrolides comprise an abundant bioactive family of the actinomycete secondary metabolites [4-6] witch exhibit antiviral [5], antibacterial [7], cytotoxic [8, 9], antifungal [10] and antitumor [11] activities. The present paper reports the usefulness of new bioassays for further investigation of bioactivity of feigrisolides, macrolides from the marine actinobacterium *Streptomyces* sp. 6167.

2. MATERIALS AND METHODS

Cultivation of bacterium and obtaining of compounds: Cultivation of bacterium *Streptomyces* sp. 6167 and extraction of Feigrisolides A, B и C described earlier [4, 12].

Obtaining of the Ehrlich carcinoma cells: Ehrlich ascite carcinoma cell were grown intra-peritoneally in white mice 18-20 g of weight. Cells were harvested on the 7^{th} to 10^{th} day after inoculation, washed twice by centrifugation (450×g, 10 min) in cold phosphate buffered saline (PBS) with following composition: NaCl – 137 мМ; KCl – 2,68 мМ; $Na_2HPO_4 \times 7H_2O$ – 8,06 мМ; NaH_2PO_4 – 1,47 мМ, pH 7.4 and finally resuspended in the PBS for the final cell concentration of $2\text{-}5 \times 10^6$ cells/ml.

Search for the inductors of apoptosis: Suspension of Ehrlich carcinoma cells (200 μl in each well of 96-well microplate included 20 μl of tested compounds) was incubated for 1 hour at 37^0C. Then 10 μl of Hoechst 33342 water solution were added to each well (final concentration of 5 μM). After 5 min of incubation at room temperature a fluorescence of cell suspension was measured at λ_{ex} = 355 nm and λ_{ex} = 460 nm using fluorescent plate reader Fluoroscan Ascent (ThermoLabsystems, Finland). At the same time the cell viability was determined using Propidium iodide as described previously. The induction of chromatin condensation (early apoptosis) was determined by comparison of fluorescence intensity of a Hoechst 33342 (apoptotic cell) and Propidium iodide (necrotic cells) in cell suspension [13].

Determination of acute toxicity *in vivo*: Male white mice weighing around 20-22g, were used in this study. The mice holding room was maintained at a temperature of 20± 3 ^{0}C, 30-70 % relative humidity and 12- h on / 12- h off light cycle. The animals were divided into three groups of five animals each. One group served as control and two other as experimental groups. The mice were intra-peritoneally given 50 mkg/ kg of studying compounds dissolved in 0,5 mL of mixture of distillated water and ethanol, except the control group, which received an equal volume of solvent only. The pathological changes (death) of animals were observed during 20 days.

Standard procedure of inhibition: Reaction mixture containing 50 µl enzyme solution (10^{-2} unit) in 0.025 M acetate buffer, pH 5.2 and 40 µl inhibitor solution (1mg/ml DMSO) was incubated at 37 °C for 10 min. After addition of 400 µl of the laminaran solution (1 mg/ml) and 20 min incubation, the residual activity of the enzyme was determinated by the corresponding method.

Enzyme assay: The activity of 1→3-β-D-glucanases was estimated by an increase in the amount of reducing saccharides determined by the method of Nelson [14]. The incubation medium contained 100 µl of the enzyme and 400 µl of laminaran solution (1mg/ml). The incubation duration did not exceed the time required for hydrolysis of 10% of the substrate contained in the incubation mixture.

Immobilization of sea urchin sperm:
Sea urchin spermatozoa were obtained using standard technique. Fertilization ability of spermatozoa is determined by their morphological, biochemical, physical prorerties. Sea urchin sperm (approximately $2x10^7$ cell/ml) and the sea water solution (1 ml) of a testing substance were exposed in glass test tubes for 1 hr. The kinetics of spermatozoa stop of movement in solutions of the testing substance was investigated by light microscopy [15].

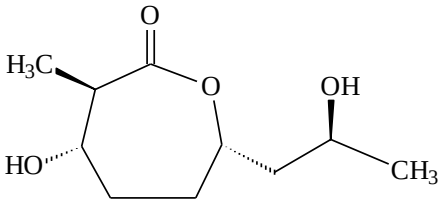
3. RESULT AND DISCUSSION

The marine isolate of actinobacterium, *Sreptomyces* sp. 6167 was found to produce known macrolide antibiotics, feigrisolides A, B, C (Fig.). The chemical structures of these substances were assigned by a combination of the 1D and и 2D NMR spectroscopy and electrospray mass spectrometry (ESIMS) [4, 12]. This report describes the results of experiments directed to testing of the biological activity of these substances. Earlier it was shown that feigrisolide B exhibits antibacterial activity against *Sporobolomyces salmonicolor* SBUG 594 (50 µg/disk) and shows weak activity against other organisms tested [4] . Feigrisolides A and C show moderate activity on Coxsackie virus B3 in the antiviral tests [4]. We have studied cytotoxic activity of feigrisolides A, B and C against tumor cells of Ehrlich carcinoma and the developing eggs of the sea urchin *Strongylocentrotus intermedius.* It was found that feigrisolide B exhibits cytotoxic effect against tumor cells and against the developing eggs at IC_{50} =3 µg/mL and IC_{50} =1 µg/mL, respectively. At the same time less hydrophobic feigrisolide A shows cytotoxic activity against these test systems at concentrations IC_{50} =20 µg/mL and IC_{50} =30 µg/mL. It was shown that feigrisolide B immobilizes sea urchin sperm in dose less then 1 µg/ml, i.e. it is spermatoxic. Additionally, a biotest based on estimating the fertilizing ability of sea urchin spermatozoids [15] showes that feigrisolide B completely blocks egg fertilization in same dose. As feigrisolides A, B, and C do not induce erythrocyte lysis at the concentration more higher than cytotoxic concentrations [12], we should suppose that they do not involve in destruction of the biological membranes and do not possess membranotropic activity [16].

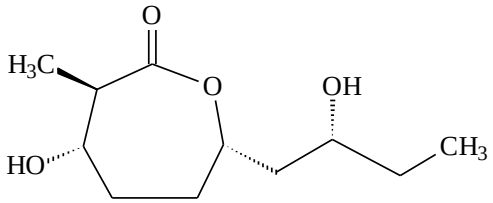
The inhibitory activities of feigrisolides A, B and C against conditional-pathogenic and pathogenic microorganisms *Proteus vulgaris* 4848, *Pseudomonas aeruginosa* H-1515, *Salmonella enteritidis* 126-6, *Staphylococcus epidermidis* 2482, *Yersinia pseudotuberculosis* H-2781, *Listeria monocytogenes* 4-B and *Staphylococcus aureus* 3515 were determined by an agar diffusion method. Feigrisolide B shown weak growth inhibition against *Listeria monocytogenes* 4-B and *Staphylococcus aureus* 3515 with MIC_{50} of 9 mM. On the other hand feigrisolide C stimulates growth of *Escherichia coli* 384 at the same concentration.

Last decade has appeared interest of studying of 1→3-*β*-D-glucanases in connection with their role in the immunity of plants and animals [17]. A few data about enzyme inhibitors from marine microorganisms and plants are in the literature [18, 19]. According to the available data follows, inhibitory activity of low-molecular weight products from marine Streptomyces have not been studied. It has been shown, that the tested macrolides are effectors of the 1→3-*β*-D-glucanase from *Spisula sacchalinensis* crystalline style. The feigrizolides A, B and C activated the action of enzyme on 50% at concentration 0.4 mM, 0.4 mM and 0.2 mM, accordingly.

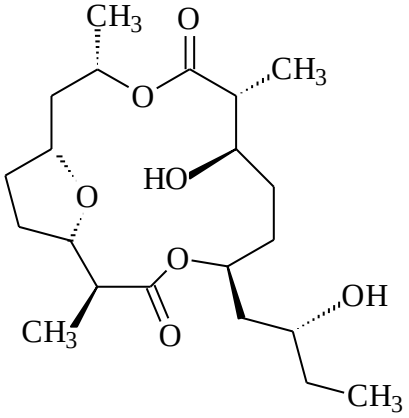
The fact that apoptosis is a physiological mechanism of cell death and has reversible stages [20] allows us to consider the possible pharmacological correction of the apoptotic process. There are many laboratories where the apoptosis activation mechanisms and different influence pathways on this process are studied as well as the search for inductors, supressors and releasers of apoptotic program are doing. According to published date, some compounds of a microbial origin induce the apoptosis, for example: verotoxins, obtaining from *E. coli*, induces apoptosis in human astrocytoma cells [21], and lovastatin from *Aspergillius terreus*, induces programmed cell death of human medulloblastoma cell lines [22]. We studied the action of the feigrisolides A, B and C on an apoptotic process in the Ehrlich carcinoma cells.. It was found that feigrisolide B induces apoptotic process with IC_{50} =17.4 µg/ml.



feigrisolide A



feigrisolide B



feigrisolide C

Figure. Metabolites of the actinobacterium Streptomyces sp. 6167.

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