BIOACTIVE COMPOUNDS

FLAVONOIDS CONTENT IN SWEET WORMWOOD (*ARTEMISIA ANNUA* L.) "HAIRY" ROOT CULTURE

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The interest in traditional medicinal plants study increased dramatically last years. This interest is associated with the new data obtained in scientific investigations of medicinal plants including their antioxidant, anti-inflammatory, antiviral, anticancer activities. The features of medicinal plants can be used for establishment of new medicines for treatment of different diseases.

Artemisia annua L. (*Asteraceae*) was the "hero" of 2015-th year Nobel Prize due to Youyou Tu discoveries concerning a novel therapy against malaria. Different compounds known as biologically active ones have been extracted from *A. annua* plants. Sesquiterpenes, flavonoids, coumarins, phenolic compounds were studied in the plants. *A. annua* extracts possess antimicrobial, anti-inflammatory, antioxidant activities etc.

"Hairy" root culture can be obtained via *Agrobacterium rhizogenes*-mediated transformation. It is considered to be an alternative way for producing valuable plant-derived compounds because these transgenic roots have been reported to synthesize large variety of secondary metabolites.

The aim of this work was to study the effect of genetic transformation on flavonoids accumulation and compared to the content of the compounds in some *A. annua* "hairy" root lines.

A. annua "hairy" roots were obtained earlier and were cultured on the hormone free half strength Murashige and Skoog basal medium. Plant material was collected after 1 month of cultivation, lyophilized and mashed into powder. The amount of total flavonoids in the 70% ethanol extracts was determined using modified Aluminium chloride colorimetric method. Rutin was used as standard and the results were expressed as rutin equivalents (RE) in milligram per gram of dry weight.

Flavonoids content in *A. annua* "hairy" root lines varied from 27.5±0.53 mg/g RE to 35.6±1.38 mg/g RE. The compounds were accumulated in transgenic roots in amount greater than in roots and leaves of the control plants. So, the genetic transformation has led to the increase of flavonoids content in *A. annua* "hairy" root lines.

Our investigation approved that transgenic roots of *A. annua* obtained via *A. rhizogene*-mediated transformation can accumulate flavonoids in greater amount than non-transformed plants. The data obtained suggest the possibility of using of *A. annua* "hairy" root culture as a source of flavonoids.

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PREPARATION, CHARACTERIZATION, PHARMACOKINETIC STUDIES, *IN VITRO* AND *IN VIVO* DELIVERY OF ARTEMISININ LOADED PCL-PEG-PCL MICELLES

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Artemisinin (ART) has anti-inflammatory, antimicrobial, antioxidant, anti-amyloid and antitumor effects, but its application is limited due to its low water solubility. To improve the bioavailability and water solubility of artemisinin, we synthesized four series of poly (ε-caprolactone) -poly (ethylene glycol) -poly (ε-caprolactone) (PCL-PEG-PCL) tri-block copolymers.

The structure of the copolymers was characterized by HNMR, FTIR, DSC and GPC techniques. ART was encapsulated inside micelles by a single-step nanoprecipitation method which leading to the formation of ART/PCL-PEG-PCL micelles. The obtained micelles were characterized by dynamic light scattering (DLS) and atomic force microscopy (AFM).

The results showed that the zeta potential of ART/ micelles was about -15.4 mV and its average size was 83.22 nm. ART was encapsulated into PCL-PEG-PCL micelles with a loading capacity of 18.62 ± 0.42% and entrapment efficacy of 89.23 ± 1.41%. The MTT assay showed that bare PCL-PEG-PCL micelles is non-toxic to MCF7 and 4T1 cancer cell lines whereas the ART/ PCL-PEG-PCL micelles showed a specific toxicity to both cancer cell lines. Pharmacokinetic study in rats revealed that *in vivo* drug exposure of ART was significantly increased and prolonged by intravenously administering ART-loaded micelles when compared with the same dose of free ART dissolved in acetone. Furthermore, *in vivo* results demonstrated that this micellar formulation significantly increased drug accumulation in tumors.

The polymeric micellar formulation of ART based on the amphiphilic block copolymer PCL-PEG-PCL could provide a desirable process for ART delivery.

A STUDY OF LIPID METABOLISM REGULATING EFFECT OF POLYPHENOLIC COMPOUNDS ISOLATED FROM GEORGIAN GRAPE SAPERAVI VARIETY ON *IN VITRO* MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases. The over-accumulation of triglycerides in the liver is a hallmark of NAFLD. The pathogenesis of NAFLD is not entirely understood. To date, the only effective treatment of NAFLD is caloric restriction (CR), which is difficult to achieve. Resveratrol – a natural polyphenol, has been shown to mimic the beneficial effect of CR, delaying the onset of a variety of age-related diseases in mammals. Recently, studies in mice found that either CR or resveratrol protected the liver from fat accumulation induced by high fat diet. However, detailed mechanisms mediating resveratrol effects remain unclear. Low grade chronic inflammation, caused by liver-resident macrophages plays an important role in pathogenesis of NAFLD. Engagement of Toll-receptors (especially TLR4) is essential for the initiation of inflammatory process. Therefore, the relationship between metabolic state and low-grade chronic inflammation is important in understanding the progression from steatosis to severe stages of NAFLD. The main aim of our study was to study an effect of different polyphenols – resveratrol, ε-viniferine and trans-piceid extracted from Georgian grape Saperavi variety, on both: lipid metabolism and inflammatory process, utilizing different *in vitro* models of NAFLD: monolayer (2D) system and three-dimensional system – hepatic spheroids.

To create an *in vitro* model of NAFLD, in case of 2D system, lipid-overload was induced by adding a mixture of oleic/palmitoleic acids to monolayer culture of either mouse hepatoma cell line – Hepa 1-6, or mouse macrophages cell line – RAW264.7 (as in the fatty liver, liver-resident macrophages are also exposed to FFA-rich environment). For the creation of 3D spheroids, Hepa 1_6 cells have been cultured in 96-well plate, covered with non-adhesive agarose gel and lipid accumulation was triggered as described above. In both 2D and 3D systems, the effect of stimulation with different concentrations of stilbenoids was assessed by flow cytometric measurement of lipid content and ROSes level, also the surface expression of TLR4 has been studied by immunophenotyping.

According to our results, in 2D cultures, a stimulation of lipid-loaded hepatocytes and macrophages with low concentration (10 μM) of both – resveratrol and trans-piceid induced a decrease in lipid accumulation and ROSes level. In case of hepatocytes, this effect was accompanied by a decrease in the surface expression of TLR4, but in macrophages the above-mentioned modulation of TLR4 surface expression has not been seen. In 3D cultures a similar effect was detected in case of higher concentrations of polyphenols (30 μM). Based on the obtained data, we hypothesize, that the molecular pathway of lipid-load reduction, caused by polyphenols, depends to some extent on the modulation of TLR4 signaling pathway.

STUDY OF NOVEL [1,10]PHENANTHROLINE BASED CYANINE DYES AS FLUORESCENT PROBES FOR NUCLEIC ACIDS

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Analysis of nucleic acids (NA) is required for a wide range of biomedical applications. One of the most used approaches for both qualitative and quantitative NA detection is based on dyes, which have initially weak emission in unbound state and strong emission when NA-bound. Cyanine dyes are known as the most effective probes for NA sensing.

Here, a series of monomethine, trimethine and styrylcyanine dyes based on novel [1,10]phenanthroline heterocycle (FT1–FT5) was synthesized and characterized as potential fluorescent probes for nucleic acids detection. The spectral properties of these dyes both in absence and in presence of dsDNA/RNA were studied by fluorescent and absorption spectroscopy. The cell staining experiments was done on HL-60 cell line using flow cytometry and fluorescent microscopy.

The [1,10]phenanthroline based cyanine dyes are weakly fluorescent (0.5-17 a.u.) in unbound state. Their excitation/emission maxima are in the ranges of 481-633 and 555-651 nm, respectively. The trimethine dyes FT4 and FT5 have comparably small Stoke shifts (11 and 18 nm, respectively), while for monomethine FT1 dye it is large (93 nm). Upon the binding to dsDNA/RNA their emission intensity rises up to 51 times (for monomethine benzothiazole derivative FT1 with RNA), excitation/emission bands shift (up to 51 nm). The strongest fluorescence intensity in complexes with dsDNA and RNA was observed for the trimethine benzothiazole derivative FT4. Quantum yield value of FT4 in complex with dsDNA is 1.5%, the binding constant is estimated as $K_b = 7.9 \cdot 10^4$ M^{-1} that is typical value of intercalating molecules. The trimethine dyes FT3 and FT4 demonstrate strong proneness to aggregation in aqueous buffer. It was shown, that the dye excess leads to formation of the dye aggregates on DNA surface. The applicability of red-emitted FT3 and orange-emitted FT4 dyes for use in flow cytometry as stains for living cells was demonstrated. Fluorescent imaging of HeLa cells by FT3 and FT4 dyes has shown that both dyes are cell permeable but target different components in the cells. FT4 stains RNA rich components – the nucleoli and most probably the cytoplasmic RNA, while FT3 stains cellular organelles located near the nuclei (probably mitochondria or lysosomes), but not the nucleic acids.

Thus, novel [1,10]phenanthroline based cyanine dyes have potency as probes for NA sensing *in vitro*; their ability to stain components of living cells is demonstrated.

APPLICATION OF DON-1R DRUG IN THE TECHNOLOGY OF LIVE FEED CULTIVATION FOR FISHES

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Searching for effective ways to improve the growth rate of the live feeds in aquaculture is a question of present interest. One among such approaches is to use substances with a stimulating effect. In pond aquaculture γ-crotonolactone-containing drug "DON-1R" is frequently used for prevention and treatment of aeromonosis. It was noticed that in addition to the therapeutic effect the drug enhances the level of natural feeds in ponds. In this regard, the possibility of DON-1R use during live feed cultivation was tested on the example of ü *Simocephalus vetulus* (Müller). The nutrient composition and hydrolytic enzymes activity of cultured organisms in addition to their productivity rate were evaluated as live feed is a source both of nutrients and hydrolytic enzymes for fish larvae.

The cultivation of *S. vetulus* was performed using yeast *Saccharomyces cerevisiae* as a feed substrate. Different concentrations of DON-1R have been applied to investigate its influence. The productivity of zooplankton's culture was defined by specific growth rate (μ/day). The content of total lipids and proteins was determined in cultivated live feed. The total lipase activity was defined by Sklyarov's unified method. The total proteolytic activity was studied at pH 4.8, 7.4 and 9.0 by Anson's modified method. Amylase activity was determined by Caraway's method.

In accordance with obtained results, the highest growth indicators of S. vetulus monoculture were noted during DON-1R application at a concentration of 66.8×10^{-6} ml/l, while the increase in the drug concentration to 100.2×10^{-6} ml/l in the cultivation medium did not result in significant rise of specific growth rate. In live feeds cultivation an important issue is avoiding the deterioration of their nutritional value during the increase in biomass accumulation rate. It was shown that the total proteins content in *S. vetulus* from the experimental and control groups did not differ significantly (513 mg/g and 576 mg/g of dry weight, respectively). Instead, crustaceans, cultivated with DON-1R, were characterized by higher total lipid content – 275 mg/g in comparison to 175 mg/g in zooplankton from the control group. Despite the established advantages of DON-1R application, it was found its inhibitory effect on enzymatic hydrolytic activity: lipase activity decreased .6 times and α-amylase – 2.7. The study of total protease activity at neutral (pH 7.4) and alkaline (pH 9.0) pH values showed its decrease 1.5 and 7 times, respectively. At the same time the proteolytic activity at acidic pH values (pH 4.8) decreased by 70 times.

Therefore, the use of DON-1R during zooplankton cultivation enhances the growth rate in *S. vetulus* monoculture, while deterioration of the nutrient composition in feed organisms is not observed. However, the use of DON-1R is accompanied by inhibition of hydrolytic activity in the feed zooplankton.

THE INHIBITORY EFFECT OF CALIX[4]ARENE C-956 ON THE PLASMA MEMBRANE Ca^{2+},Mg^{2+}-ATPase OF UTERINE MYOCYTES

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Plasma membrane Ca^{2+},Mg^{2+}-ATPase (PMCA) functions as a fine tuner of cellular calcium concentration and plays a pivotal role in the termination of Ca^{2+} signal in uterine myocytes. Regarding its critical contribution to the maintenance of Ca^{2+} homeostasis, this enzyme can be an important pharmacological target. It was shown that calix[4]arene C-90 (100 μM) decreased PMCA activity by 75% with no significant influence on the activity of other plasma membrane ATPases. Indeed, other calixarene derivatives can be more effective inhibitors of PMCA, and calix[4]arene C-956 could be one of them.

The effect of different calix[4]arenes (C-715, C-772, C-960, C-716, C-957, C-975, C-90, C-956) on PMCA activity as well as the influence of calix[4]arene C-956 on membrane ATPases (Mg^{2+}-ATPase, Ca^{2+}-ATPase, Na^{+},K^{+}-ATPase) were tested in plasma membrane fraction isolated from pig's myometrium. Using confocal microscopy and laser correlation spectrometry, we studied the effect of calix[4]arene C-956 on $[Ca^{2+}]_i$ in uterine myocytes loaded with Ca-sensitive probe Fluo-4 AM and its effect on the effective hydrodynamic diameter of myocytes, respectively.

The first our task was to determine the most efficient inhibitor of PMCA activity among above-mentioned calixarenes. Using enzymatic assay, we demonstrated that calix[4]arene C-956 had the most prominent inhibitory effect on specific enzyme activity ($I_{0.5}$ = 15.0 ± 0.5 μM, n = 5). In order to test selectivity of calix[4]arene C-956 the specific enzymatic activity of membrane ATPases was determined in the presence of 100 μM calixarene in the incubation medium. The results showed that this calixarene decreased specific plasma membrane Ca^{2+},Mg^{2+}-ATPase activity by 79.2% but had no statistically significant influence on other ATPase activities proving selectivity of calix[4]arene C-956 action.

It was also demonstrated that application of 20 μM calix[4]arene C-956 into uterine myocytes caused a temporary increase in intracellular Ca^{2+} concentration. Interestingly, during 2.5 minutes $[Ca^{2+}]_i$ decreased that could be explained by involvement of compensatory mechanisms which regulate calcium homeostasis. In addition, 50 μM calix[4]arene C-956 induced a decrease in myocyte's hydrodynamic diameter by 45% that could be due to contraction of myocytes.

Thus, calix[4]arene C-956 is a selective inhibitor of PMCA which is more effective than other calixarenes and therefore is a promising compound for modulation of smooth muscle contractility.

THE EFFECT OF BACTERIOPHAGAL DSRNA ON ARGININE METABOLISM OF RAT MICROGLIAL CELLS EXPOSURED TO HYPOXIA *IN VITRO*

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Larifan is a pharmaceutical product containing a heterogeneous population of natural origin double-stranded RNA (dsRNA). It is an agonist of TLR3. Larifan exhibits an antineoplastic effect by the modulation of functions of immune cells including macrophages. Tumor hypoxia dramatically influences tumor-associated macrophage functions and shifts their metabolism to alternative (M2) phenotype, which is characterized by the decrease of the production of inflammatory mediators including NO. *In vitro* studies have shown that hypoxic M2 macrophages can be reprogrammed by the use of TLR agonists to promote tumor regression. It is accepted that glioma associated macrophages are characterized by the restricted capacity to shift their metabolic profile in response to polarizing agents. The aim of the work was to investigate the effect of Larifan on arginine metabolism in rat microglial cells (MC) exposed to hypoxia *in vitro.*

Rat MC were cultured under normoxic (21% O_2) or hypoxic (3% O_2) conditions for 24 h. After this cells were treated with Larifan (at the concentrations of 200 μg/ml) or Larifan+bacterial LPS (50 μg/ml) for 18 h in normoxic and hypoxic conditions, respectively. To characterize arginine metabolism nitrite level was assayed by the Griess reaction, arginase activity was measured in cell lysates by colorimetric method.

The 24 h exposure of MC to hypoxia *in vitro* led to the 2-fold increase of MC arginase activity along with the decrease of NO generation as compared to normoxic cells. Larifan used alone moderately stimulated arginase activity of both normoxic and hypoxic MC and did not influence their NO production. Used in combination with bacterial LPS Larifan intensified the inhibitory effect of the latter on arginase activity of hypoxic MC. Stimulatory effect of LPS on NO production by hypoxic MC was hampered by Larifan.

Thus, hypoxia shifted arginine metabolism in MC to arginase pathway. Larifan synergized with bacterial LPS to decrease arginase activity in hypoxic MC, but it interfered with the stimulatory effect of LPS on NO generation in these cells.

MAGHEMITE (γ-FE_2O_3) NANOPARTICLES AS A POTENTIAL SORBENT OF EXTRACELLULAR GLUTAMATE IN SYNAPTIC CLEFT

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In the mammalian central nervous system, amino acid and its form glutamate play a primary role as a key excitatory neurotransmitter. Changing its extracellular concentration in the synaptic cleft it is possible to control physiological state of brain. Thus, in this work attention was paid to interaction of γ-Fe_2O_3 NPs with glutamic acid on the level of model in similar buffer solution which used for nerve brain terminals isolation and further dimensional analysis of formed aggregates.

Interaction of NPs with glutamate was occurred in distilled water and buffer solution. For sorption determination was used radiolabeled L-[^{14}C] glutamic acid. Different concentrations of NPs were used for determination concentration dependent correlation. After mixing glutamate solution with NPs, it was centrifuged 15 min at 13,400 rpm. With help of scintillation counter sorption was measured in the aliquots of the supernatants by liquid scintillation counting with scintillation cocktail ACS and was expressed as percentage of total amount of radiolabelled neurotransmitter absorbed.

For the investigation of NPs' behavior in different media was chosen size change determination. The function of particle's size distribution was investigated with the help of laser correlation spectrometer ZetaSizer3 Malvern Instrument.

These nanoparticles show quite good ability to glutamate sorption at different concentrations, the average sorption of glutamate by nanosized particles at concentration of 3 mg/ml is 94%.

Mean value of intact NPs' size is 171 nm. Mean value of NPs' size is 156 nm. 0.5 nM solution of L-[^{14}C]glutamic acid didn't cause aggregation. After addition of NPs solution into media containing different chelating agents, sizes significantly increased. Mean value of NPs' size is 564 nm.

It has been found during experiment that nanoparticles elicit sorptive properties towards glutamate and in distilled water sorption equals 94% at NPs' concentration 3 mg/ml. However, it becomes seriously limited in work buffer solution due to the presence of NaH_2PO_4 and to a lesser degree HEPES that are the necessary constituents. Thus, it should be find out more convenient biological medium for synaptosoms storage and NPs sorption ability study *in vitro*. Dimensional analysis has shown that NPs in standard salt solution form huge aggregates and do not work correctly. Small amounts of glutamate do not cause changing in sizes.

GOLDEN ROOTS PLATFORM: THE *IN VITRO* GENETICALLY ENGINEERED CULTURES FOR ARTEMISININ PRODUCTION IN *ARTEMISA SIEBERI*, *A. DIFFUSA* AND *A. BIENIS*

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WHO recommends artemisinin-based combination therapies (ACTs) as the most effective choice to treat malaria. As artemisinin cannot be synthesized chemically in an economically feasible way due to their complex chemical structure, we suggest genetically transformed root cultures as an alternative production system. High stability of the production of secondary metabolites is an interesting characteristic of hairy root cultures.

We used the leaves of one month sterile plants as explants in inoculation medium of *Agrobacterium rhizogenes* strains A4, ATCC15834, MSU440, and A13 (MAFF-02-10266) for 6 min. The explants were transferred to a modified co-cultivation MS mediums for 48 h. The explants were placed on MS medium supplemented with 400 mg/l cefotaxime. The genomic DNA was extracted from transformed root tissue using CTAB DNA isolation method in order to show the integration of T-DNA of *A. rhizogenes* in transgenic roots. Molecular analysis of transformed root lines was confirmed by PCR using specific primers of the *rolB* gene.

The results showed a significant increase in transformation frequency when the strain MSU440 was used. Artemisinin content in genetically transformed root cultures was detected by HPLC analysis.

In the present study, an efficient hairy root induction system for *Artemisia sieberi*, *A. diffusa* and *A. bienis* was developed through *Agrobacterium rhizogenes*-mediated transformation as an alternative approach for artemisinin production.

DIRECT ORGANOGENESIS AND *IN VITRO* REGENERATION OF MEDICINAL PLANT *PLANTAGO LANCEOLATA*

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Plantago lanceolata is a medicinal plant which has valuable secondary metabolites including iridoid glycosides, phenylpropanoid glycosides, flavonoids and phenylcarboxylic acids. The iridoid glucosides Aucubin and Catalpol are the main constituents which have biological activities such as anti-inflammatory (as a specific inhibitor of NF-ƙB) and cytotoxic activity. Efforts in the molecular regulation of secondary metabolites biosynthesis are restricted by the lack of efficient protocols for plant regeneration. In this study, we succeeded in *in vitro* regeneration of this plant.

Leaf, hypocotyl and root explants excised from *in vitro P. lanceolata* were cultured on Murashige and Skoog (MS) medium supplemented with various plant growth regulators benzylaminopurine (BA), α-naphtalenacetic acid (NAA) and 3-indolyl acetic acid (IAA) at different concentrations. The obtained direct and adventitious shoots were transferred to root induction medium containing 0.1 mg/l IBA for 10 days. The plantlets were placed to free hormone MS medium for more growth.

Callus induction was obviously appeared on most of explants after 2 sub-cultures. After two weeks, shoot organogenesis appeared in shoot induction media. The highest regeneration frequency was obtained using MS medium containing MS medium including containing 2 mg/l BA and 0.5 mg/l NAA. It was revealed that hypocotyl and root explants are better than leaf explants.

The present protocol prepares a simple and rapid regeneration system for *P. lanceolata* in short period via direct and adventitious shoot induction. *In vitro* regeneration could be used as a strong tool in genetic transformation to produce transgenic medicinal plants.

ANTIBACTERIAL EFFECT OF *PFAFFIA PANICULATA* AGAINST SOME PATHOGENIC BACTERIA

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Pfaffia paniculata (belonging to *Amaranthaceae* family) is named Brazilian ginseng which plays a major role in the revival of traditional medicine. Despite the useful role of its root in many ailments such as anti-diabetes, anti-ulcer and anti-cancer, we investigated anti-bacterial activities of roots of *P. paniculata* against some strains of bacteria.

The antibacterial activity of extracts of *P. paniculata* was assessed by disc diffusion method on *Bacillus cereus*, *Kelbsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi* and *Escherichia coli*. Extract powder was solved in dimethyl sulfoxide (DMSO) and then filtered. Antibacterial effects of the extracts tested at different concentrations (2.5, 5, 10, 20, 40, 60, 80 and 100 mg/ml). Kanamycin (1 and 10 mg/ml) and DMSO was used as positive and negative controlrespectively.

Our results revealed that ethanolic extracts have an inhibitory effect on *E. coli* and no effect on other bacteria. Three concentration (60, 80 and 100 mg/ml) of *P. paniculata* extracts showed significant inhibitory activity in compared with antibiotics. The mean of inhibitory of three concentration (10, 11 and 12.5 mm of inhibition zone) was more than kanamycin at 1 mg/ml concentration (8.5 mm) but were not better inhibitory than kanamycin at 10 mg/ml concentration (21 mm) on *E. coli*.

So we can conclude that this plant has antibacterial properties. The result can be related to the nature of the compounds found in this plant. Based on this result, *P. paniculata* can be effective at higher doses for the control of *E. coli*.

PREPARATION OF S- AND R-ENANTIOMERS OF 3-ACETOXY-7-BROMO-5-PHENYL-1,2-DIHYDRO-3H-1,4-BENZODIAZEPIN-2-ONE USING PIG LIVER CARBOXYLESTERASES

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Carboxylesterases (EC 3.1.1.1) are the most studied enzymes, catalyzing the enantioselective hydrolysis of an exceptional range of acyclic, carbocyclic and heterocyclic compounds, including 3-hydroxy-1,4-benzodiazepin-2-one esters. Nevertheless, the limitations of the carboxylesterase usage are the high cost of commercial enzyme and its single usage. Therefore, the application of more economical partially purified carboxylesterase as a component of pig liver microsomal fraction, as well as development of its immobilization on polymeric carriers are urgent tasks.

The aim of the present work was the isolation of carboxylesterase preparations from the pig liver, their immobilization on polymeric carriers and studying their biochemical and physico-chemical features to conduct enantioselective hydrolysis of 3-acetoxy-7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one.

An accessible method of immobilization of pig liver microsomal fraction in agar was developed. The biocatalyst with 90% preservation of the initial esterase activity and widening of the pH-optimum of the immobilized preparation (pH 6.0-8.0) was obtained.

Enantioselective hydrolysis of the studied compound using immobilized microsomal fraction was conducted in optimized reaction conditions. The S-enantiomer of substrate was obtained ([α]20D = +116.9, c = 1 in chloroform). The biocatalyst was used for the enantioselective hydrolysis of the ester for 5cycles of usage in a batch process.

According to the modified method, cytosolic carboxylesterase was isolated from the pig liver.

The belonging of the enzyme to the carboxylesterase family was indicated by the complete inhibition of its activity by the selective carboxylesterase inhibitor bis(p-nitrophenyl) phosphate and the results of electrophoresis.

It was shown, that the regioselectivity of the obtained protein fractions was significantly different. The specific activity of the most active with β-naphthyl acetate fraction (Rf 0.05) was 64 times higher than its α-naphthyl acetate hydrolytic activity.

Using the isolated cytosolic carboxylesterase, under the developed conditions, enantioselective hydrolysis of 3-acetoxy-7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one with obtaining of R-enantiomer of substrate ([α]20D = +117.2, c = 1 in chloroform) was conducted. This indicates an opposite enantioselectivity of the pig liver cytosolic carboxylesterase compared with the carboxylesterase of the microsomal fraction.

NEW INHIBITORS OF TYROSINASE

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Melanin pigmentation of skin plays the most important role in the protection of organism against UV-irradiation, capable to induce serious pathological states, including cancerous diseases of skin. But the excessive accumulation of melanin brings to toxic melanodermia, melasma, lentigo and other skin lesions and is the important current dermatological and cosmetological problem.

Tyrosinase (EC 1.14.18.1) is the key enzyme of skin melanin pigment biosynthesis. In spite of certain progress in investigation of natural and synthetic tyrosinase inhibitors, the urgency of such studies is high, because the existing inhibitors are in some cases unstable, expensive, toxic, require complex methods of synthesis or isolation from natural sources.

Tyrosinase of *Agaricus bisporus* was isolated according to modified method. It was found, that addition of PEG-4000 during extraction promotes the 3-fold decrease of polyphenols content. It is known, that the products of endogenous polyphenolic compounds oxidation are inhibitors of tyrosinase, their removal allowed increasing tyrosinase activity by 25%.

The search of new inhibitors of tyrosinase among the wide range of compounds, including derivatives of benzoic acid, 3-chloro-1,4-naphthoquinone, dipicolinic acid, etc. The studied substances did not display the inhibitory effect at concentration of 0.1-0.5 mmol/dm^3.

It is known, that the natural substrate of mushroom tyrosinase is 1,8-dihydroxynaphthalene, thus it was supposed that the 2,7-dihydroxynaphthalene may be a promising inhibitor of enzyme activity.

It was shown, that the concentration of half-maximal inhibition (IC_{50}) of tyrosinase monophenolase activity by 2,7-dihydroxynaphthalene (96.5 μmol/dm^3) is close to that of kojic acid (60.75 μmol/dm^3) – a classic inhibitor of melanogenesis. It was found, that 2,7-dihydroxynaphthalene exerts inhibitory action only on monophenolase activity of tyrosinase in contrast to kojic acid, which inhibits both monophenolase and diphenolase enzyme activity.

For the first time the influence of hydroxybenzylidene aminophenols on tyrosinase activity was studied. It was found, that 4-hydroxybenzylidene-4-aminophenol and 2-hydroxybenzylidene-2-aminophenol possess the inhibitory ability, which 5- and 23-fold (IC_{50} – 11.2 and 2.6 μmol/dm^3, respectively) exceeds that of kojic acid. The studied hydroxybenzylidene aminophenols similarly to 2,7-dihydroxynaphthalene, influence only the monophenolase activity of enzyme.