ИНСТИТУТ ПО ОРГАНИЧНА ХИМИЯ
С ЦЕНТЪР ПО ФИТОХИМИЯ
ГОДИШНА НАУЧНА СЕСИЯ ПОСВЕТЕНА НА 150^{ТИЯ}
ЮБИЛЕЙ НА БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ

17–18 АПРИЛ 2019 г.

ИОХЦФ БАН

150 ГОДИНИ БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ 1869

ПРОГРАМА

17.04.2019 г.

10.00 Проф. д-р П.Долашка ННП „Иновативни нискотоксични биологично активни средства за прецизна медицина“ (БиоАктивМед)
10.30 Гл. ас. д-р Б. Трушева ННП „Здравословни храни за силна биоикономика и качество на живот“
11.00 Проф. д-р М. Попова „Преход към възобновяема енергия: ще намалим ли емисиите на CO2?“
13.30 Постерна сесия

18.04.2019 г.

10.00 Проф. дхн В. Банкова "Опазване на Европейското биоразнообразие чрез оползотворяване на традиционното знание за билките за разработване на иновативни продукти"
10.30 Проф. дхн Т. Цончева "Възможности за обучение и трансфер на знания в областта на екологията и иновативните материали по програма Хоризонт 2020"
11.00 Проф. д-р П. Шестакова и доц. д-р Б. Стамболийска „Национална Научна Инфраструктура ИНФРАМАТ“
13.30 Постерна сесия

15.30 Награждаване на най-добрите постери

ГОДИШНАТА НАУЧНА СЕСИЯ, ПОСВЕТЕНА НА 150ТИЯ ЮБИЛЕЙ НА БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ, ПРОВЕДЕНА НА 17-18 АПРИЛ 2019 г. Е ОРГАНИЗИРАНА С ЛЮБЕЗНОТО СЪДЕЙСТВИЕ НА:

ЧЛЕН-КОРЕСПОНДЕНТ ПРОФ. ИВАН ПОЖАРЛИЕВ

И

ПРОЛАБ ИНСТРУМЕНТС ЕООД

АСМ2 ЕООД

ШИМАДЗУ БЪЛГАРИЯ ЕООД

СОФЛАБ ООД

ИНСТИТУТ ПО ОРГАНИЧНА ХИМИЯ С ЦЕНТЪР ПО ФИТОХИМИЯ

ГОДИШНА НАУЧНА СЕСИЯ ПОСВЕТЕНА НА 150ТИЯ ЮБИЛЕЙ НА БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ

ПОСТЕРНА СЕСИЯ – РЕЗЮМЕТА

EFFECT OF VITAMINS AND PLANT GROWTH REGULATORS TREATMENTS ON CAFFEOYLQUINIC ACIDS IN *INULA BRITANNICA* SHOOT CULTURES

Antoaneta Trendafilova, Milka Todorova, Miroslav Rangelov, Kalina Danova

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

Caffeoylquinic acids (CQA) are phenylpropanoid compounds, produced by the plant organism as a part of its disease-resistance responses to biotic and abiotic stress. In the human organism these compounds have been found to possess a wide array of beneficial pharmacological properties such as antioxidant, antibacterial, anticancer and antihistamic activities.

The aim of the present work was to elaborate the effect of tissue culture media optimization on the production of different CQA derivatives in a model system of shoot cultures of the medicinal plant *Inula britannica*.

For this purpose the combined effect of Murashige and Skoog (MS) vs. Gamborg (G5) vitamin supplementations with low (0.2 mg/l) and high (0.7 mg/l) concentrations of benzyl adenine (BA), applied alone or in combination with 0.1 mg/l naphthylacetic acid (NAA) were studied. The content of caffeoylquinic acids was studied by means of HPLC analysis of the methanol extract of the *in vitro* cultured plant.

It was established that the different vitamin supplementations affected differentially the content of total CQA in relation to the plant growth regulators (PGR) applied. Thus, while in the PGR-free control and in media, in which BA was applied alone, the MS vitamins resulted in enhanced CQA production as compared with the G5 supplementation. On the contrary, application of NAA in combination with BA reversed this dependency. In all treatments chlorogenic and 3,5-dicaffeoylquinic acids were the predominant components. The 0.2 mg/l BA supplementation was most favorable in stimulation of 3,4-dicaffeoylquinic acid in both vitamin supplementations. And MS vitamins favored the production of 1,5- and 4,5-dicaffeoylquinic acid in the PGR-free control and media, supplemented with BA.

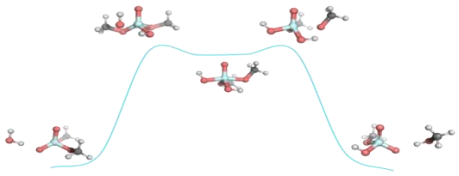
Recent studies reveal the potential of the 3,5- and 1,5- dicaffeoylquinic acids in the treatment of neurodegenerative diseases. The obtained results are of interest in obtaining fundamental knowledge on the factors affecting the targeted biotechnological delivery of selected CQA-derivatives with pharmacological potential in *in vitro* culture model system of *Inula britannica.*

Acknowledgements
The authors are thankful to the National Scientific Fund, Bulgaria (DN 09/11)

DFT/PCM STUDY ON THE HYDROLYTIC STABILITY OF PHOSPHATE AND ARSENATE MONO-, DI- AND TRIESTERSTERS

S. Dobrev[1,2], D. Cheshmedzhieva[1], S. Angelova[2], T. Dudev[1]
[1]*Faculty of Chemistry and Pharmacy, University of Sofia "St. Kliment Ohridski", 1 James Bourchier Blvd, 1164 Sofia, Bulgaria*
[2]*Institute of Organic Chemistry with Centre of Phytochemistry, Acad. G. Bontchev str. Bl. 9, 1113 Sofia, Bulgaria*

In 2011 Wolfe-Simon *et al.* [1] showed experimental results on bacteria growing in arsenate-rich environment suggesting that arsenic could be an efficient substituent for phosphorus in organism's DNA. Although the scientific community largely discredited these results, the study drew the scientists' attention on the possibility of arsenic substituting for phosphorus in biomolecules. The main argument against this is the higher rate of hydrolysis (i.e. lower hydrolytic stability) of arsenate esters (mimicking arsenate DNA backbone) as compared to the respective phosphate counterparts. This conclusion, however, has been based the reactions of mono- or triesters conducted in alcoholic solutions. Those conditions do not match the intracellular milieu. Considering the impact of such a possibility on exobiology, we took it upon ourselves to conduct an extensive computational study of the hydrolysis of phosphate and arsenate esters simulating biological conditions. The aim of this work is to provide some theoretical insights into the mechanism of hydrolysis of the monoanions of mono- and dimethyl esters of phosphate and arsenate, as well as neutral trimethyl esters in water and in 1-propanol (mimicking DNA or DNA/partner protein low-dielectric environment). Calculations have been done at B3LYP/6-311++G(2d,2p) level of theory, using PCM implicit solvation model. The mechanism of the hydrolytic reaction is associative and involves two transition states.
A comparative analysis of the results indeed shows lower activation energies for all arsenate esters in respect to the phosphate ones. Activation energies for both transition states are identical and increase with the size of the esters. For monomethyl esters we got 44.9 and 28.9 kcal/mol for phosphate and arsenate respectively, for dimethyl esters - 46.3 and 30.1 kcal/mol, and for trimethyl esters - 49.4 and 31.5 kcal/mol. This means that the difference of activation energies between esters of the same species is less than 5 kcal/mol for phosphates and less than 3 kcal/mol for arsenates. The dielectric media does not significantly change the energy profile for mono- or diester hydrolysis, as has been reported by Mladek *et al.* [2] for various monoesters.



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TRITERPENOIDS AND PHYTOSTEROLS IN *INULA ENSIFOLIA* L.

Eli Kostadinova, Angel Konakchiev, Milka Todorova, Antoaneta Trendafilova

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Inula ensifolia L. is one of the species of genus *Inula* (Asteraceae) distributed in Bulgaria. Literature data for this species are scarce and revealed the presence of thymol derivatives in the roots, as well as tocopherol, two norisoprenoids, three quercetin derivatives and four caffeoylquinic acids in the aerial parts [1]. Although the plant has no any documented medicinal use, an antiproliferative activity of methanolic extract from *I. ensifolia* against human cancer cell lines in vitro has been reported [2]. As a part of ongoing project on *Inula* species growing in Bulgaria, we have studied chemical constituents in the aerial parts of *Inula ensifolia* L.

Aerial parts of the plant were collected from Slavyanka Mt, air-dried and extracted with chloroform at room temperature. The resulting chloroform extract was separated by CC on Silica gel and the obtained subfractions were analyzed by GC/MS and/or ^{1}H NMR. Campesterol, stigmasterol, β-sitosterol, α- and β-amyrin, lupeol, taraxasterol and ψ-taraxasterol, and the corresponding 3-O-acetates and 3-O-palmitates as well as 3-*O*-palmitates of 16*β*-hydroxylupeol, 16β-hydroxy-β-amyrin, faradiol and arnidiol were identified by GC/MS, ^{1}H NMR and/or TLC comparison with authentic standards.

Structure elucidation of the palmitate esters was confirmed by acid hydrolysis, separation and characterization of the triterpene alcohols and diols by GC/MS. The presence of palmitic acid was proven by GC/MS after methylation. All of the triterpenoid components are now reported for the first time in the investigated plant.

Acknowlegement: This work was supported by the National Science Fund, Ministry of Education and Science, Bulgaria, project DN 09/11.

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SUPER-CRITICAL CARBON DIOXIDE AS EFFECTIVE ECO-FRIENDLY SOLVENT FOR EXTRACTION OF PURSLANE (*PORTULACA OLERACEA* L.)

Sabina Taneva, Svetlana Momchilova, Angel Konakchiev, Vladimir Dimitrov

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

The aim of this study was to evaluate the extraction of purslane with super-critical CO_2 concerning the yield at different conditions (temperature, pressure) and to compare the results to that of three common classic methods for plant extraction (of Folch, of Bligh and Dyer, and with hexane in Soxhlet apparatus).
Purslane (*Portulaca oleracea* L.) was collected in August 2017 in the region of Novo Zhelezare (near Plovdiv). Stalks and leaves were finely chopped, dried at room temperature in shadow, then ground finely and subjected to extraction. Portions of 800 g were used for super-critical CO_2 experiments at different temperatures (55 and 80°C) and pressures (100, 255, 400 bar). 15 g were extracted by the method of Folch (with chloroform-methanol 2:1 mixture), of Bligh and Dyer (with chloroform-methanol 1:2 mixture) and in Soxhlet extractor (with hexane, 6 hours). The yield was determined gravimetrically.
The experiments with super-critical CO_2 revealed that increased temperature and pressure improved the yield. On the other hand, the three classic methods applied ensured yield of about 4% which was lower than that of 6.2% achieved by super-critical CO_2 at 80°C and pressure of 400 bar.
Extraction of purslane with super-critical CO_2 ensures higher yield than that of the common methods which use solvents as hexane and chloroform-methanol mixtures. Moreover, the super-critical CO_2 is undoubtedly the preferred solvent being non-toxic, non-corrosive, non-flammable and cheap.

Acknowledgements: Financial support provided by the Program for supporting of young scientists and PhD students at the Bulgarian Academy of Sciences – 2017, Project ДФНП 17-60, is gratefully acknowledged. The equipment for super-critical CO_2 extraction is purchased by Grant BG161PO003-1.2.04-0007-C0001 under OP Development of the Competitiveness of the Bulgarian Economy 2007-2013.

The poster was presented at 11th SEMINAR OF ECOLOGY- 2018 WITH INTERNATIONAL PARTICIPATION 26-27 April 2018.

FATTY ACIDS COMPOSITION OF PURSLANE (*Portulaca oleracea* L.) EXTRACTED BY SUPER-CRITICAL CO_2

S. Taneva, S. Momchilova, Y. Nikolova, A. Konakchiev, D. Antonova, V. Dimitrov

Institute of Organic Chemistry with Centre of Phytochemistry – Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

Purslane (*Portulaca oleracea* L.) is a herbaceous succulent annual plant of the Portulacaceae family, which is common in Europe, Africa, North America, Australia and Asia. According to the World Health Organization this species is one of the most widely used medicinal herbs because of its antibacterial, anti-inflammatory, antioxidant, antidiabetic, hypocholesterolemic properties. In the Mediterranean, as well as in some other European and Asian countries, it is consumed mainly as a fresh salad, dish or spice.

Purslane is one of the richest sources of the essential 18:3 ω-3 fatty acid (α-linolenic acid) among the green leafy plants. Until now, various procedures with different solvents (hexane, chloroform, methanol, 2-propanol, dichloromethane, acetone, etc.) or their mixtures have been used for extraction of lipids from leaves and stems of that widely spread herb but no data about application of the non-toxic, eco-friendly and cheap super-critical carbon dioxide (SC-CO_2) have been found in the literature. Therefore, the aim of our study was to examine the potential of this green technology for extraction of lipids from purslane. For the purpose, fatty acid composition of SC-CO_2 extracts at different conditions was determined in order to investigate effects of pressure and temperature on the product quality. Moreover, fatty acids composition of SC-CO_2 extracts was compared to that obtained by hexane [1] and chloroform-methanol [2,3] procedures. Finally, the fatty acid composition of purslane leaves and stems was compared to that of seed oil.

Acknowledgements: Financial support provided by the Program for supporting of young scientists and PhD students at the Bulgarian Academy of Sciences – 2017, Project ДФНП 17-60, is gratefully acknowledged. The equipment for super-critical CO_2 extraction is purchased by Grant BG161PO003-1.2.04-0007-C0001 under OP Development of the Competitiveness of the Bulgarian Economy 2007-2013.

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The poster was presented at 11th CHEMISTRY CONFERENCE 11 – 13 October 2018.

MONITORING FOR POLUTANTS IN DUMPS FROM OPEN-PIT MINING OF MARITSA IZTOK BASIN: POLYCYCLIC AROMATIC HYDROCARBONS

Stefan Marinov[1], Maya Stefanova[1], Zlatka Milakovska[2], Achim Bechtel[3]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Geological Institute, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3] *Montan universität, Department of Applied Geosciences and Geophysics, Leoben, Austria*

Polycyclic aromatic hydrocarbons (PAHs) in the environment are directly related to the health and quality of life. Their presence is referred to the development of various diseases. Hence, monitoring environmentally harmful compounds is of great importance for the life of humans and animals. Releases of organic compounds from dump materials could be a result of natural and anthropogenic weathering processes [1, 2].
The aim of the present study is to determine qualitatively/quantitatively PAHs in Maritsa Iztok basin dump materials, Bulgaria. PAHs in six dumps samples were under consideration. Gas chromatography-mass spectrometry (GC-MS) was used to appreciate organic matter sources and to identify and quantify PAHs in samples. Total organic carbon (TOC) values of the samples varied in the range 1.18-5.63 wt. %. Total PAHs amounts of the samples were in the range of 7–52 microg/kg. The set of PAHs determined pointed out 3 and 4 ring unsubstituted PAHs as dominant in the dump extracts. The values measured were compared with the norms for PAHs in soils in Bulgaria (Regulation No 3/01.08.2008). All magnitudes were under the regulation norms, approaching the background values for PAHs in soils.
In conclusion, the study is an equivocal proof for the negligible amounts of PAHs in dump materials from Maritza Iztok lignite mining. However, their amounts in dumps should be measured and monitored bearing in mind the huge territories covered by these waste materials.

Acknowledgment: The financial support of NSF (Ministry of Education and Science, Bulgaria) in the frame of DN 04/5-2016 project is highly acknowledged.

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BRANCHED ALKANES WITH QUATERNARY CARBON ATOMS (BAQAs) ISOLATED FROM MARITSA IZTOK DUMP MATERIALS

Maya Stefanova[1], Zlatka Milakovska[2], Stefan Marinov[1]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Geological Institute, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Three pseudohomologous series of branched alkanes with one quaternary carbon atom (BAQCs; C_{18}-C_{37} range) were registered for the first time in the extractable organic matter of dump materials from Maritsa Iztok lignite mines. By SIM the following BAQCs series were tracked: *m/z* 85 - 3-Ethyl,3-Methyl alkanes (3E3MAs), "even" numbered, nC$_{18}$- nC$_{30}$; *m/z* 99 - 3,3-Diethyl alkanes (3,3-DiEAs), "odd" numbered, nC$_{19}$-nC$_{29}$, and, *m/z* 127 – 5,5-Diethyl alkanes (5,5-DiEAs), "even" numbered, nC$_{19}$-nC$_{37}$. The last one homologs were preponderant in all samples studied. These pseudohomologous families share the particularity of including isomers with exclusively "odd" or "even" carbon numbers, clearly indicating a biosynthetic origin [1].

BAQCs such as 5,5-DEAs and 3,3-DEAs are widespread in sediments and sedimentary rocks (ranging from 2.1 billion years to present-days), that could be explained by their very low biodegradability [1].

There were some attempts to use BAQCs as a proxy for environmental estimate. Bai *et al.* [2] correlated 5,5-DiEAs isolated from soils with the spatial distributions of climate and vegetation. The results demonstrated that the distribution of 5,5-DEAs can serve as a new independent proxy, indicating variations in ecosystem/climate, just as the proxy nC$_{15}$-nC$_{21}$ *vs.* nC$_{22}$-nC$_{33}$ ratio developed for *n*-alkanes. Lower molecular weight 5,5-DEAs maximizing at nC$_{19}$ or nC$_{23}$ were attributed to warm/wet climate, while high molecular weight 5,5-DEAs maximizing at nC$_{29}$ were preponderant in cold, arid palaeoenvironments. According to this assumption, the 5,5-DiEAs distribution pattern centered at nC$_{23}$ in samples studied unequivocally confirm their affection to mild continental climate [3] typical for NE Thrace.

Acknowledgment: The financial support of NSF (Ministry of Education and Science, Bulgaria) in the frame of DN 04/5-2016 project is highly acknowledged.

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STUDENTS INTERNSHIPS RAISESEE PROJECT: PREPARATION AND APPLICATION OF ACTIVATED CARBON FROM MIXTURE OF CHERRY STONES AND WALNUT SHELLS

Стоил Цолов, Славчо Абрашев, Карина Бозова, Йоана Стойкова
119 SU "Akad. M. Arnaudov", Latinka str. Bl11, Sofia.

Radostina Ivanova, MiglenaVasileva, Silvia Hristova, NartzislavPetrov, Liudmil Antonov and Tanya Tsoncheva

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, "Akad. G. Bontchev " str. Bl 9, Sofia.

Current investigation is focused on the preparation of activated carbon from mixture of cherry stones and walnut shells. The texture and surface properties were tested by iodine number, titration by Böhm method and FTIR spectroscopy. The adsorption properties of the activated carbon were investigated in water purification from methylene blue using UV-Vis spectroscopy. The activated carbon was modified by $Cu_{0.5}Zn_{0.5}Fe_2O_4$ using incipient wetness impregnation with methanol solution of the corresponding nitrates. The catalytic properties of the obtained modification were tested in methanol decomposition with a potential of hydrogen production.

Acknowledgements: RAISESEE project No 17167 for financial support and Institute of Organic Chemistry with Centre for Phytochemistry, Bulgarian Academy of Sciences, for the opportunity for students training is acknowledged.

STUDENTS INTERNSHIPS RAISESEE PROJECT: PREPARATION AND APPLICATION OF ACTIVATED CARBON FROM CHERRY STONES

Melisa Rudeva, Vladimir Doichinov, Petar Simeonov, Tamar Hadi

119 SU "Akad. M. Arnaudov", Latinka str. Bl11, Sofia.

Radostina Ivanova, MiglenaVasileva, Silvia Hristova, NartzislavPetrov, Liudmil Antonov and Tanya Tsoncheva

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, "Akad. G. Bontchev " str. Bl 9, Sofia.

Current investigation is focused on the preparation of activated carbon from cherry stones from Bulgarian canning industry. The texture and surface properties were tested by iodine number, titration by Böhm method and FTIR spectroscopy. The adsorption properties of the activated carbon were investigated in water purification from methylene blue using UV-Vis spectroscopy. The activated carbon was modified by $ZnFe_2O_4$ using incipient wetness impregnation with methanol solution of the corresponding nitrates. The catalytic properties of the obtained modification were tested in methanol decomposition with a potential of hydrogen production.

Acknowledgements: RAISESEE project No 17167 for financial support and Institute of Organic Chemistry with Centre for Phytochemistry, Bulgarian Academy of Sciences, for the opportunity for students training is acknowledged.

COPPER-MODIFIED COAL ASH ZEOLITES AS HETEROGENEOUS CATALYSTS FOR VOCs OXIDATION

Hristina Lazarova[1]*, Silviya Boycheva[2], Denitza Zgureva[2], Margarita Popova[1]

[1]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 9, 1113 Sofia, Bulgaria, *lazarova@orgchm.bas.bg*
[2]*Technical University of Sofia, Department of Thermal and Nuclear Power Engineering, 8 Kl. Ohridsky Blvd., 1000 Sofia, Bulgaria*

Volatile organic compounds (VOCs) are the main class of air pollutants, emitted from various industrial processes. Since their release into the atmosphere can cause serious environmental problems, the treatment of VOCs is essential for the control of air quality and for the protection of the environment. Thermal and catalytic oxidations, biological treatment and the adsorption processes have widely been used to remove VOCs. Catalytic oxidation can significantly reduce energy costs and the formation of harmful by-products, such as carbon monoxide or nitrogen oxides, and it is one of the most attractive ways to control the emissions of VOCs. Various types of catalysts were applied for the total oxidation of VOCs but the development of highly active and cheap catalyst is still an open area. Fly ash zeolites (FAZ) are a promising alternative to the already developed catalysts. In contrast to the natural and synthetic zeolites they consist of ferrous oxides (γ-Fe_2O_3, α-Fe_2O_3, γ-Fe_3O_4) and traces of Cu, Co, Mn, V, W, etc., which is a prerequisite for their excellent catalytic activity. The catalytic activity of FAZ can be improved further by modification with metal oxide species.
In this study Cu-modified fly ash zeolites were synthesized and studied in the total oxidation of model mixture of VOCs (n-hexane, acetone, toluene, 1,2 dichlorobenzene).
The initial fly ash zeolites (FAZ) were synthesized by alkaline atmospheric crystallization (AA) and double stage fusion-hydrothermal activation (FHA) of FA samples collected from the two biggest Thermal Power Plants in Bulgaria, namely (TPP "Maritza East 2" (ME2) and TPP "AES Galabovo" (AES). The obtained zeolites were characterized by X-ray diffraction studies and were assigned as a crystalline phase of zeolite Na-X. They possess high specific surface values (SBET, m^2/g) calculated from their nitrogen adsorption isotherms. Copper-modified fly ash zeolites (Cu-FAZ) were prepared by incipient wetness impregnation technique with copper acetylacetonate. The loading of 5 wt. % copper on the zeolite samples was achieved. The relationships between the surface properties, concentration and state of iron and copper oxide species and catalytic activity of Cu-FAZ in total oxidation of VOCs (n-hexane, acetone, toluene and dichlorbenzene) were studied. Copper-modified FAZ show high catalytic activity in VOCs oxidation, Cu-FAZ_AES_FHA material being the most active. The conversion above 80 % in the temperature interval 553-723 K was registered on Cu-FAZ_AES_FHA for two VOCs molecules - n-hexane and 1,2 dichlorobenzene. Stable catalytic activity for the degradation of the mixture of VOCs was registered for Cu-FAZ_AES_FHA showing negligible deactivation for the studied VOCs molecules even in 3 reaction cycles.

Acknowledgements Financial support from the Bulgarian Scientific Fund (grant ДН 17/18) and the Bulgarian-Hungarian Inter-Academic Exchange Agreement is greatly appreciated.

CONFORMATIONAL STUBILITY OF *HELIX ASPERSA* HEMOCYANIN

Yuliana Raynova[1], Svetla Todinova[2], Ivan Angelov[1], Krassimira Idakieva[1]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Hemocyanins (Hcs) are oligomeric copper-containing glycoproteins that function as oxygen carriers in the hemolymph of several mollusks and arthropods [1]. Besides their important biological function, mollusk Hcs have shown promising properties in the development of various medicinal products including antiviral agents, conjugate vaccines and immunotherapeutic agents for cancer [2, 3]. One of the first steps, required to outline the design of therapeutic properties of protein drugs, is their thermodynamic characterization.
Conformational stability of the hemocyanin, purified from garden snail *Helix aspersa* (HaH), was investigated using a number of spectroscopic techniques and differential scanning calorimetry (DSC).
The pH range 6.5 – 8.0, characterized by the absence of pH-dependent fluorescence changes, was defined as a pH-stability range of the Hc molecule. Scattering experiments showed that in TRIS-HCl buffer, pH 8.0, HaH molecules are predominantly in monomeric state. DSC studies demonstrated the considerable thermal stability of HaH (T_m ~ 80 ^oC). Thermal denaturation of HaH was described by the two-state irreversible model and parameters of the Arrhenius equation were calculated. Study of the thermal unfolding of HaH by means of circular dichroism showed that the Hc was structurally and functionally stable up to 75 °C. Buffers were found to have specific interactions with the protein, resulting in either a destabilizing effect or a promotion of stability and increase of denaturation temperature. Stabilizing effect of both TRIS and HEPES buffers was established. It was found that salt and sugar additives at optimal concentration 100 mM contribute to the stability of the Hc.
Received data will allow creating stabilized Hc formulation to facilitate the further investigation of therapeutic properties and applications of this dioxygen-binding protein.

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NANOSTRUCTURED MESOPOROUS CERIA-TITANIA COMPOSITES DOPED WITH COPPER AS CATALYSTS FOR HYDROGEN PRODUCTION

Aleksandra Mileva and Tanya Tsoncheva

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, "Akad. G. Bontchev " str. Bl 9, Sofia.

The aim of the current investigation is to prepare mesoporous ceria-titania binary materials using template-assisted hydrothermal technique. All materials were modified with copper (8 wt.%) by incipient wetness impregnation with aqueous solution of $Cu(NO_3)2.9H_2O$. The obtained materials were studied by a complex of physicochemical techniques such as N_2 physisorption, XRD, UV-Vis, FTIR and Raman spectroscopies and TPR with H2. Their catalytic activity was tested in methanol decomposition to CO and hydrogen with a potential application in alternative fuels. Modification of ceria and/or titania oxides with copper promotes their catalytic activity in methanol decomposition to syngas, but this effect is strongly influenced by support composition.

Acknowledgements:
This work was supported by Bulgarian National Scientific Fund (Grant Number КП-06-H29/2). Project BG05M2OP001-1.002-0019:„Clean technologies for sustainable environment – water, waste, energy for circular economy"(Clean&Circle), for development of a Centre of Competence is also acknowledged.

* E-mail: aleksandra_bunalova@abv.bg

REMOVAL OF PHENOLS FROM WATER BY POLYMER-BASED CARBON

A. Bahova, M. Vasileva, G. Georgiev, G. Mehmed, T. Budinova, I. Stoycheva, B. Petrova, N. Petrov

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

Removal of penthachlorophenol, aminophenol and nitrophenol from water by activated carbon synthesized on the base polymer wastes was investigated. Activated carbons were produced by termo-oxidation treatment of different polymer wastes and subsequent carbonization and hydro-pyrolysis. The obtained activated carbons are distinguished by high surface area and microporous structure. Carbon adsorbents demonstrated relatively high adsorption capacity towards phenolic compounds. Adsorption capacity of thus synthesized activated carbons is related to the surface area and composition of the prepared material, as well as to the nature of the adsorbent.

All three adsorbates show high affinity towards carbon, confirming that the retention mechanism occurs via non-specific interactions between the electronic density of the adsorbent and the aromatic pollutants. Electrostatic interactions may also appear depending on the solution pH and the charge distribution of the carbons.

SYNTHESIS, CHARACTERIZATION AND APPLICATION OF NANOPOROUS CARBONS FROM BIOMASS

G. Georgiev, M. Vasileva, A. Bahova, T. Budinova, I. Stoycheva, B. Petrova, N. Petrov, B.Tsyntsarski

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

Nanoporous carbons using different biomass wastes as precursors were synthesized. Suitable treatment was found for conversion of wastes from the production of juices into activated carbons with high surface area. Using precursors with high lignin content lead to production of activated carbons containing pores of a larger size, while precursors with high cellulose content yields activated carbon with a predominantly microporous structure. The obtained activated carbons are suitable for removal of highly toxic cadmium ions from aqueous solution. The extent of removal of metal ions is determined by the porous structure of the carbon, size and chemical nature of the surface.

APPLICATION OF NATURAL DEEP EUTECTIC SOLVENTS FOR GREEN EXTRACTION OF BIOACTIVE COMPOUNDS FROM POPLAR PROPOLIS: A PRELIMINARY STUDY

Hristo Petkov, Boryana Trusheva, Milena Popova, Vassya Bankova

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

At present one of the key issues in the chemistry field is the development of so called "green" technologies that aims to preserve the environment and to reduce the negative influence of human involvement. Among the diverse ways of “green” technology, developing and applying “green” solvents are one of the most important subjects. Recently, a new type of “green” solvents, named deep eutectic solvents (DESs), was developed. DES is a mixture of proton acceptor and a proton donor in solid state, which are joined by intermolecular hydrogen bonds. The resulting mixture is eutectic, i.e. it has a lower melting point than that of any of its individual components and it is liquid even at very low temperatures. In a particular combination and ratio natural compounds in solid state (primary metabolites) may also be converted into liquids to form so called natural deep eutectic solvents (NADESs), which are present in nature. They are biocompatible and play a role in all kinds of cellular processes of living organisms.

NADESs have several advantages as solvents: easy and inexpensive preparation, biodegradability and in addition, the precursors used for their production are natural, non-toxic, renewable compounds, which are abundant in our daily foods. It was found that NADESs have a number of interesting applications in electrochemistry, functional materials, organic synthesis, catalytic transformations and pretreatment processes. Recently, the interest in NADESs increases due to their successful application in the extraction of bioactive plant metabolites, including non-water soluble. Besides, the extracted compounds can be easily recovered from NADES. This predicts a great potential for NADESs as solvents in the extraction processes of valuable secondary metabolites, further implemented in the food or pharmaceutical industry.

Still the most effective and widely used way for exhaustively extraction of all biologically active components from poplar type propolis, is extraction with 70% ethanol in water. The use of alcohol, however, limits the application of propolis as it is contraindicated in a number of diseases, in young children and pregnant women. In order to protect both human health and the environment it is necessary to seek alternative ways of extraction of this valuable biologically active complex. Therefore the present study aims to apply for the first time a new approach for extraction of biologically active compounds from poplar type propolis based on the use of NADESs and to evaluate their extraction efficiency.

Acknowledgment: The financial support of the National Science Fund of Bulgaria, project DN19/4, is gratefully acknowledged.

Представено като постер на 2nd Propolis Conference “Propolis in Human and Bee Health Conference”, Sofia, Bulgaria, 28-29.09.2018

CHEMICAL CHARACTERIZATION AND RADICAL-SCAVENGING ACTIVITY OF PROPOLIS FROM PITCAIRN ISLAND

Boryana Trusheva, Kristina Georgieva, Rosa Dimitrova, Milena Popova, Vassya Bankova

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria

Propolis (bee glue) is a resinous hive product collected by bees from certain plant sources and known since ancient times for its healing properties. There is a number of scientific evidence that it has antimicrobial, antioxidant, anti-inflammatory, immunomodulating, antiulcer, anti-diabetic and anticancer activities. Because of its broad spectrum of biological activities there is an undying interest on the composition of propolis, which depends on the vegetation of the area of collection. Numerous studies on the chemical composition and biological activity of propolis from Europe, South America, Asia and the Pacific region exist in the literature however there is no data about propolis from Pitcairn Islands. In the last years it is of growing commercial interest, because Pitcairn's bee population is disease-free and their products are considered as clean of pollutants.

The aim of this work was to determine volatile and non-volatile composition as well as radical-scavenging activity of propolis from Pitcairn Island (South Pacific Ocean), which is not investigated yet.

The essential oil were obtained by hydrodistillation extraction of raw propolis in a Likens-Nickerson type apparatus and then analyzed by GC/MS. The main volatile components were monoterpenes p-mentha-1,5-dien-8-ol (4.4 %) and α-pinene (3.4 %), and sesquiterpenes β-caryophyllene (3.3 %), caryophyllene oxide (2.4 %) and verbenone (2.3 %). The major non-volatile constituents according to the GC/MS analysis after silylation were terpenoids (mainly diterpenes and less triterpenes). Phenolic components (cardanols, alk(en)ylresorcinols and anacardic acids) are present in significantly lower amounts, as flavonoids and the usually present phenolic acids are completely absent. On the basis of the overall chemical profile, hypotheses were made regarding the plant sources of the sample.

DPPH radical scavenging activity of propolis ethanol extract was tested and the results showed week activity.

Acknowledgements: The authors would like to thank to Pitcairn Island Producers' Co-operative (PIPCO) for providing the sample.

Представено като постер на
10th Conference on Medicinal and Aromatic Plants of Southeast European Countries (CMAPSEEC), Split, Croatia, 20-24.05.2018

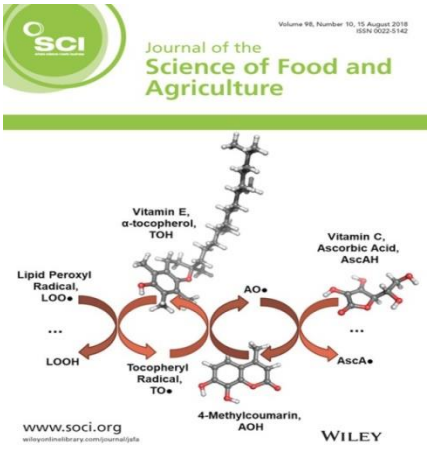
PROTECTIVE EFFECTS OF NEW ANTIOXIDANT COMPOSITIONS OF 4-METHYLCOUMARINS AND RELATED COMPOUNDS WITH DL-***α***-TOCOPHEROL AND L-ASCORBIC ACID*,**

Vessela D. Kancheva[1], Adriana K. Slavova-Kazakova[1], Silvia E. Angelova[1], Luciano Saso[2], Ashok K. Prasad[3], Virinder S. Parmar[3,4]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Department of Human Physiology and Pharmacology "Vitorio Erspamer", University of Rome "La Sapienza", Rome, Italy*
[3] *Department of Chemistry, University of Delhi, Delhi, India*
[4] *Institute of Advanced Sciences, Dartmouth, MA, USA*

Coumarin derivatives possess a wide range of biological activities. By functionalization of the parent coumarin skeleton that has neither antioxidant nor biological activity, a series of new bio-antioxidants has been designed. New antioxidant compositions (equimolar binary and ternary mixtures) of eight 4-methylcoumarins and three related compounds have been created, tested and different effects between individual components have been observed: synergism (positive effect), additivism (summary effect) and antagonism (negative effect). The highest oxidative stability of the lipid substrate was obtained in the presence of the new antioxidant compositions of the studied compounds with DL-***α***-tocopherol and L-ascorbic acid. The role of each component in the antioxidant compositions of ternary mixtures has been identified by using new equations composed for the first time by the authors. All ternary mixtures demonstrate synergism as a result of continuous regeneration of DL-***α***-tocopherol from the studied antioxidants and L-ascorbic acid. Theoretical calculations have been probed as indicators of the expected effects between the individual components in a binary mixture.

Acknowledgements: The calculations have been performed on the computer system installed at IOCCP-BAS with the financial support of the Bulgarian National Science Fund under Project MADARA (RNF01/0110, contract N_DO02-52/2008) (*decommissioned*).



***** Published in
J. Sci. Food Agric. (2018), 98(10): 3784-3794,
DOI 10.1002/jsfa.8892.
** The cover image of *J. Sci. Food Agric.* (2018), Volume 98 (issue 10) is based on this research article;
https://onlinelibrary.wiley.com/toc/10970010/2018/98/10

NEW HYBRID PHOTOCATALYTIC AGENTS FOR PHOTODYNAMIC METHOD TOWARDS MICROBIAL DRUG RESISTANCE

Vanya Mantareva[1], Meliha Aliosman[1], Ivan Angelov[1], Yavor Mitrev[1], Mahmut Durmus[2], Vesselin Kussovski[3]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Gebze Technical University, Dept. of Chemistry, Gebze, Turkey*
[3] *The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

The drug resistance of pathogenic microorganisms to the conventional chemotherapeutic agents features as a major challenge to the scientific community. One very prospective approach to fight the microbial resistance appears the photodynamic inactivation (PDI) with new generation photoactive drugs [1]. PDI or antimicrobial PDT takes action due to the interaction of a photoactive compound irradiated with proper gentle light in oxygen atmosphere. The final result is singlet oxygen and other cytotoxic oxygen species, all toxic to living cells.
The study presents new type hybrid materials developed on the bases of a phthalocyanine photosensitizer and a natural biologically-active compound among steroids, the both with functionality during the photodynamic process. These hybrid molecules can act by combining the unique optical properties of phthalocyanine macromolecule and physiological action of sterol molecule so that working together they appear multifunctional and target specific photosensitizers.
Acknowledgements: Support by the project **KP-06-29/11** of the National Science Fund, Bulgaria.

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IN SILICO CONSTRUCTION AND REFINEMENT OF MT1 MELATONIN RECEPTOR

Nadezhda Todorova[2], Violina T. Angelova[3], Jana Tchekalarova[4], Miroslav Rangelov[1]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3] *Faculty of Farmacy, MU-Sofia, Sofia, Bulgaria*
[4] *Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Molecular simulations of membrane proteins, that form more than 50% of drug targets based on their key role in signaling pathways, in a native-similar bilayer environment provide atomic-scale insights into their function that are not easily obtainable in any other way. Melatonin, the neurohormone of the pineal gland, acts in humans via G protein-coupled membrane receptors, MT1 and MT2, expressed in various areas of the central nervous system and in peripheral tissues. Melatonin displays exceptional multiple complexities of actions, the basis of its integrative role by which it is distinguished from many other important signal molecules. Melatonin receptor (MT1) is an attractive target for elaboration of new drug candidates, but unfortunately is known to be unstable out of the membrane lipid bilayer, which makes the obtaining of a crystal structure by X-Ray difraction (XRD) an elusive goal. Up to now, there is no published real structure suitable for docking of new ligands targeting this receptor. However, there are lots of model based on the data from crystallized rhodopsin, but they are too artificial for reasonable docking of drug-like candidate molecules. To overcome these drawbacks, we build an in silico molecular model of melatonin receptor in membrane bilayer in water cell, with explicit water molecules. For that purpose, we used GROMACS molecular mechanics software with GROMOS force field and TYP3P water model. Calculations were carried out in periodic boundary conditions at 300 K and one bar pressure, Physisological NaCl content and pH7. By the simulation, we caught the act of melatonin entering the receptor which enlightened a wide variety of interactions that can facilitate or to disturb the movement of melatonin to the hardly accessible active site of MT1. Molecular docking of the drug like candidates was performed on receptor model. The information can be used along with data obtained from the structure of melatonin-receptor complex to construct new analogs of melatonin, capable not only to activate the receptor but also to successfully manage their way to the MT1 binding site.

PRODUCTION, STRUCTURAL ELUCIDATION AND *IN VITRO* ANTI-TUMOR ACTIVITY OF TREHALOSE LIPID BIOSURFACTANT FROM *RHODOCOCCUS WRATISLAVENSIS* STARAIN

Proletina Kardaleva[3], Biliana Nikolova[1], Iana Tsoneva[1], Severina Atanasova[1], Lyudmila Kabaivanova[2], Nelly Christova[2], Lilyana Nacheva[2], Ivanka Stoineva[3]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences Sofia, Bulgaria;*
[2]*Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria;*
[3]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria.*

Biosurfactants are naturally occurring surface active biomolecules, owing the amphiphilic character, produced by microorganisms. Amphiphilic trehalose lipids with anti-cancer properties can be useful tool for identification of structural features of glycoconjugates in the process of drug development [1].
Rhodococcus wratislavensis was selected as a producer of the biologically active substance of interest. Strain BN38 was found to produce glycolipid biosurfactant on n-hexadecane as the sole carbon source. The biosurfactant was purified using medium pressure liquid chromatography and characterized as trehalose lipid tetraester (THL) by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS).
The cytotoxic potential of trehalose lipid was evaluated by MTS viability test [2] for 24 and 48h on two different types of cancer cells (MCF7-low metastatic and MDA-MB231 high metastatic) to test its specificity. Different concentrations of the trehalose lipids were applied to prove that its action depended on it.
Initial data for the mechanism of antitumor effect of the purified trehalose lipids determined its potential for continuing the investigations with method of electroporation.

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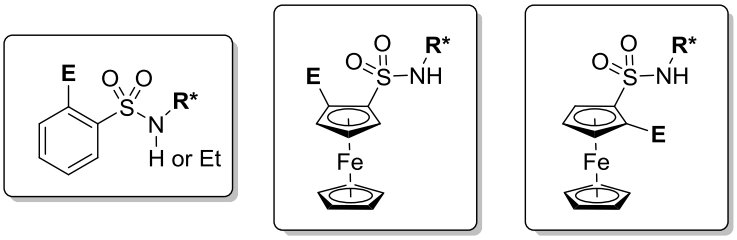
Acknowledgment: Thanks are due to the Grant DN 11/2 of the National Fund for Scientific Research, Republic of Bulgaria.

EFFICIENT SYNTHESIS OF BENZENE AND FEROCENE SULFONAMIDES POSSESSING ANTIMYCOBACTERIAL ACTIVITY

M. Ravutsov[1], Zh. Petkova[1], G. Dobrikov[1], V. Valcheva[2], V. Dimitrov[1]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

The treatment of *Mycobacterium tuberculosis* remains a significant problem due to the observed multi-drug resistance. The preparation of new, more effective agents with potential anti-tuberculosis activity is object of considerable synthetic efforts. Many compounds possessing sulfonamide moiety are notably used to treat a wide range of pathogenic microorganisms, such as *Mycobacterium tuberculosis* [1], *Escherichia coli*, *Salmonella*, *Enterobacter species* [2], *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus niger* [3], *etc.*
Ferrocene and its derivatives have been recognized as suitable molecules for biological applications because of the stability of the ferrocenyl group in aqueous and aerobic media and its favorable electrochemical properties [4]. Remarkably, there is no data in the literature concerning the biological activity of ferrocene sulfonamides. Recently, we have investigated several (+)-camphor derivatives bearing ferrocenylmethylidene and sulfonamide moieties that have shown promising cytotoxic and cytostatic activities against a large set of cancer and normal human cell lines [5].
Herein we present a synthetic approach for preparation of new chiral benzene and ferrocene sulfonamides derivatives. Selected compounds were evaluated for *in vitro* antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and have shown excellent anti-mycobacterial activity. The most active compounds were studied against multi-resistant strains.



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PHOTOCATALYTIC PROPERTIES OF ZINC OXIDE/POLYSTYRENE NANOCOMPOSITE FOR REMOVAL OF REACTIVE BLACK 5 DYE

Katerina Zaharieva[1], Silvia Dimova[2], Irina Stambolova[3], Mariya Kyulavska[2], Filip Ublekov[2], Ljubomir Dimitrov[4]

[1] *Institute of Catalysis, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Institute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3] *Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4] *Institute of Mineralogy and Crystallography "Acad. Ivan Kostov", Bulgarian Academy of Sciences, Sofia, Bulgaria*

The photocatalytic behavior of zinc oxide/polystyrene nanocomposite in the reaction of photocatalytic degradation of Reactive Black 5 dye (5 ppm) in aqueous solution under UV irradiation was studied.
The zinc oxide/polystyrene nanocomposite was obtained by mixing hydrothermally synthesized zinc oxide with commercial polystyrene solution in toluene. The Fourier transform infrared spectroscopy, powder X-ray diffraction analysis and Atomic force microscopy (AFM) were used to determine the structure, phase composition and morphology of prepared samples. AFM analysis shows also a good distribution with a monodisperse nature of the received ZnO nanoparticles. AFM images obtained verified the spherical morphology of the ZnO nanoparticles featured with raspberry-like-shaped surfaces.
The performed photocatalytic investigations show that the degree of degradation of Reactive Black 5 dye after 120 minutes UV illumination and using zinc oxide/polystyrene photocatalyst is higher (84%) in comparison with that in the presence of zinc oxide photocatalyst (74%).

A NEW APORPHINE-BENZYLISOQUINOLINE ALKALOID FROM *LEPTOPYRUM FUMARIOIDES* L. (RANUNCULACEAE)

Radostina Toshkovska[1], Tsvetelina Doncheva[1], Nadezhda Kostova[1], Amgalan Solongo[2], Yadamsuren Gerelt-Od[2], Stefan Philipov[1]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Institute of Chemistry and Chemical Technology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia*

Leptopyrum fumarioides L. belongs to the family Ranunculaceae is distributed throughout Siberia, Mongolia, China and North Korea. The areal parts of the plants are used in Mongolian and Tibetan folk medicine for the treatment of fever, typhoid fever, increased blood pressure, liver, cardiovascular and gastrointestinal diseases, dropsy and for treatment of various
intoxications [1, 2]. It is well known that the plants belonging to the family Ranunculaceae are rich in alkaloids [3]. As a result of a phytochemical study of *L. fumarioides* growing in Mongolia, a new dimeric alkaloid was isolated. Its structure was elucidated by spectroscopic methods including 1D NMR, 2D NMR, and ESIMS. To our knowledge, this is the first example for aporphine-benzylisoquinoline alkaloid with two ether bridges which have „head-to-head“ and „tail-to-tail“ coupling.
Furthermore, the previously reported for *L. fumarioides* alkaloids leptopyrine and protopine were also obtained. Their structures were elucidated by direct comparison of their spectral data with authentic sample.

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PHYTOCHEMICAL INVESTIGATIONS OF TUNISIAN *PISTACIA LENTISCUS* FRUIT CAKE, LEAVES AND FRUIT

Firas Feki[1], Antoaneta Trendafilova[2], Angel Konakchiev[2], Vladimir Dimitrov[2], Sami Sayadi[1]
[1] *Center of Biotechnology of Sfax, Laboratory of Environmental Bioprocesses, Sfax, Tunisia*
[2] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*

The *Pistacia lentiscus L*. is an evergreen member of the Anacardiaceae family consisting of nine species and five subspecies. It is largely distributed in the Mediterranean basin Tunisia, Algeria, Morocco, Spain, France, Italy, Greece, Turkey, etc. [1]. The plant is widely used in folk medicine to treat various diseases (hypertension, ulcer, eczema and diarrhea) as well as to flavor and conserve diverse aliments [2, 3]. In addition, polar extracts were found to exhibit antioxidant, anti-inflammatory, cytoprotective and anticancer activities and they were also effective inhibitors of α-amylase, α-glucosidase, lipase, and acetylcholinesterase [2, 3]. The essential oil obtained from the aerial parts of *P. lentiscus* exhibited appreciable antibacterial, antifungal and insecticidal activities. *Pistacia lentiscus* L. fruit is a source of edible oil, containing a considerable amount of unsaturated fatty acids, carotenoids and tocopherols, natural antioxidants and essential fatty acids. The residue (fruit cake) obtained from *Pistacia* fruit oil extraction by cold pressing is generally thrown out in the nature. The re-use of this agro-industrial residue to find extracts rich in bioactive compounds as essential oil, fatty acids, or more polar compounds with high added value is innovative.

The aim of the present study was to evaluate the yields of the essential oil, polar and non-polar compounds extracted from *Pistacia* fruit cake compared with that from fruit. In addition, the volatile compounds in the leaves of *P. lentiscus* were also investigated.

The essential oils were prepared by a micro distillation-extraction in a Likens-Nickerson apparatus from cake, fruit and leaves. The composition of the essential oils was analysed by GC-MS. The extraction with different solvents (hexane, chloroform, ethyl acetate and methanol) of non-polar and polar compounds was also investigated. The obtained yields for fruit cake were compared with those obtained from fruit and leaves.

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Acknowledgment: This work is carried out within the project EXANDAS (EXploitation of Aromatic plaNts' by-proDucts for the development of novel cosmeceuticAls and food Supplements; duration: 2016-03-01 to 2020-02-29. This project has received funding from the European Union's H2020-MSCA-RISE-2015 under grant agreement No 691247. The EXANDAS team is grateful for the support.

SYNTHESIS, MOLECULAR STRUCTURE AND ANTIOXIDANT ACTIVITY OF NEW BENZIMIDAZOLE FORMAZANS

M. Argirova[1], K. Anichina[2], D. Yancheva [1], S. Stoyanov[1], A. Mavrova[2], M. Kondeva-Burdina[3]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *University of Chemical Technology and Metallurgy, Sofia, Bulgaria*
[3] *Department "Pharmacology, Pharmacotherapy and Toxicology", Medical University, Sofia, Bulgaria*

The chemistry of formazans have attracted the interest of many research groups due to their wide biological activity such as antioxidant, antiviral, antimicrobial, anti-inflammatory, anti-cancer, anti-HIV [1], [2], [3].

Herein we report the synthesis of new formazan derivatives containing benzimidazole fragments obtained by autoxidative coupling of 2-hydrazino-1*H*-benzimidazole in pyridine solutions with a variety of benzimidazole hydrazones. Tautomerization properties and geometric isomers based on conjugate π-system of formazans were theoretically studied using density functional theory (DFT) methods. It was found that according to the electronic and steric properties of the substituent at the C3 position, the azohydrazone chain adopts either closed-ring chelate configuration or half-closed ring configuration.

Selected formazans were investigated for hepatoprotective and antioxidant activity on isolated rat hepatocytes. In tert-butyl hydroperoxide induced oxidative stress, the studied compounds showed cytoprotective and antioxidant effects similar to those of Quercetin. Different possible mechanisms of antioxidant action such as hydrogen atom transfer (HAT), single-electron transfer (SET-PT), sequential proton loss electron transfer (SPLET) were estimated by DFT computations in polar and nonpolar medium.

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Постера е представен на Summer School „Supramolecular chemistry in Medicine and in Technology: Advances and Challenges”, гр. Албена, България в периода 30.08.2018 – 03.09.2018.

IMMUNOMODULATING POLYSACCHARIDES FROM THE LEAVES OF THE BALKAN RESURRECTION ENDEMIC PLANT *HABERLEA RHODOPENSIS* FRIV. (ORPHEUS FLOWER)

Yordan N. Georgiev[1], Gabriela A. Fazlova[2], Berit S. Paulsen[3], Manol H. Ognyanov[1], Christian W. Wold[3], Petko N. Denev[1], Kari T. Inngjerdingen[3], Frode Rise[4], Hilde Barsett[3], Albert I. Krastanov[2], Tsvetelina G. Batsalova[5], Balik M. Dzhambazov[5]

[1]*Laboratory of Biologically Active Substances - Plovdiv, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Plovdiv, Bulgaria*

[2]*Department of Biotechnology, University of Food Technologies, Plovdiv, Bulgaria*

[3]*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, Oslo, Norway.*

[4]*Department of Chemistry, University of Oslo, Oslo, Norway*

[5]*Department of Developmental Biology, Plovdiv University Paisii Hilendarski, Plovdiv, Bulgaria*

The ethnopharmacological use of *Haberlea rhodopensis* Friv. has stimulated investigations to prove the detoxifying, restorative and rejuvenating effects of its leaf extracts by evaluation of their antioxidant, radioprotective, immunomodulating, anti-aging and wound healing properties. The research on the plant is still restricted to some standard metabolites and antioxidant enzymes, but studies on the structure and bioactivity of its polysaccharides are missing. Therefore, the aim of the current work was to investigate the chemical diversity and immunomodulating potential of water-soluble polysaccharides from the leaves of *H. rhodopensis*. By using boiling water extraction, after removal of alcohol-soluble molecules from the leaves and a combination of anion-exchange and size-exclusion chromatography, several polysaccharide fractions were obtained. On the basis of GC-MS glycosidic linkage analyses and 2D NMR studies, was revealed that the leaves contain water-soluble highly methoxylated and low acetylated homogalacturonan-rich pectins with a smaller highly ramified rhamnogalacturonan type I (RG-I) region rich in arabinogalactan II (AG-II) structures and RG-II. The endotoxin free purified polysaccharides expressed potent *in vitro* macrophage-activating ability and *ex vivo* stimulating effects on T-cell subpopulations, NK cells and granulocytes from peripheral human blood. It was proposed that the polysaccharides could act through the toll-like receptors on macrophages by using comparative studies with IFN-γ, Pam3 and lipopolysaccharides. It was demonstrated that the enzyme-released and chromatographically purified RG-I with AG-II structural motifs and RG-II regions induced higher NO production from macrophages than the initial pectic fraction with a prevalence of RG-I activity. Therefore, these highly ramified structures contained some of the responsible antigenic epitopes for the observed effect. It was concluded that the pectins in *H. rhodopensis* are at least partly responsible for its restorative activity expressed as immunomodulating effects. Furthermore, a polysaccharide-rich extract with high polyphenol content and ORAC antioxidant activity was prepared and could be used as a source of antioxidant and immunomodulating compounds.

Acknowledgements

This work was supported by the Program for support of young scientists and doctoral students in the Bulgarian Academy of Sciences – 2017 [grant № DFNP-17-62/26.07.2017].

CO-PIGMENTATION OF BLACK CHOKEBERRY (*ARONIA MELANOCARPA*) ANTHOCYANINS WITH PHENOLIC CO-PIGMENTS AND HERBAL EXTRACTS

Daniela Klisurova, Ivalina Petrova, Manol Ognyanov, Yordan Georgiev, Desislava Teneva Maria Kratchanova, Petko Denev

Laboratory of Biologically Active Substances, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Plovdiv, Bulgaria

The co-pigmentation of black chokeberry (*Aronia melanocarpa*) anthocyanins with ten phenolic co-pigments was studied. Tested compounds provoked different co-pigmentation effect, manifested by hyperchromic and batochromic shifts. The co-pigmentation was accompanied by a magnification of color intensity and decrease of color hue, both related to a more pleasant color. The hyperchromic effect was the most significant for rosmarinic acid (51.02%), syringic acid (43.24%) and catechin (39.73%). However, it was observed at the highest pigment/co-pigment ratio (1:50), not achievable in plant matter. Targeting the potential practical application of co-pigmentation, we tested eight herbal extracts for their co-pigmentation ability with aronia anthocyanins. The use of herbal extracts led to a significant hyperchromic effect at much lower pigment/co-pigment ratios, compared to pure compounds. The use of selected herbal extracts as co-pigments opens realistic prospects for development of aronia functional foods with improved sensory properties and biological effects, due to enhanced color and anthocyanin stability.

Acknowledgements: This work was funded by the National Science Fund, Bulgarian Ministry of Education and Science (Grant number DN 09/20 from 21.12.2016).

IN VITRO ASSESMENT OF THE ANTIOXIDANT ACTIVITY OF NEW BENZIMIDAZOLE-2-THIONE HYDRAZONE DERIVATIVES AND DFT STUDY OF THEIR MECHANISM OF ACTION

Neda Anastassova[1], Denitsa Yancheva[1], Nadya Hristova-Avakumova[2], Vera Hadjimitova[2]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Department of Medical Physics and Biophysics, Faculty of Medicine, Medical University-Sofia, Bulgaria*

Benzimidaozle-2-thione derivatives containing hydrazone moiteties designed as melatonin analogues were effective in inhibiting induced oxidative stress and acted as potent hepatoprotectors [1]. As a continuation from our previous work we have synthesized new derivatives of the benzimidazole-2-thione containing residues of vanillin, syringaldehyde and veratral.
For the estimation of the antioxidant potential of the tested compounds have been chosen two model systems containing stable free radicals (ABTS and DPPH) and one evaluating protection effect against ferrous iron induced oxidative damage of lecithin. The studied compounds demonstrated different extent of scavenging effect against both stable free radicals which could be attributed to their structural dissimilarity and to the different mechanism of radical neutralization (HAT for DPPH and SET for ABTS).
All compounds demonstrated capability to diminish the concentration of the ABTS radical. Comparison of the estimated using linear regression analysis C-50 values for the vanillin and syringaldehyde containing compounds denoted stronger activity than the reference Trolox at the same experimental conditions. The third compound containing veratral had weaker antiradical capacity against.
In the DPPH model system no statistically significant decrease of the absorbance of the samples containing veratral residue compared to the control ones was observed. Again we witnessed better antiradical effectiveness of the hydrazones with vanlllin and syringaldehyde moiety compared to the Trolox.
All the tested compounds decreased ferrous iron induced oxidative molecular damage. For all the hydrazones the observed protection effect was in the same concentration range like the one of strong reference antioxidants as Trolox and Quercetin.
Different possible mechanisms such as hydrogen atom transfer (HAT), single-electron transfer (SET-PT), sequential proton loss electron transfer (SPLET) were studied by DFT computations of the respective reaction enthalpies in polar and nonpolar solvents. The reactivity against various free radicals was accounted by analyzing the thermodynamic data of reactions with hydroxyl, hydroxyperoxyl, alkoxyl and alkoxyperoxyl radicals.

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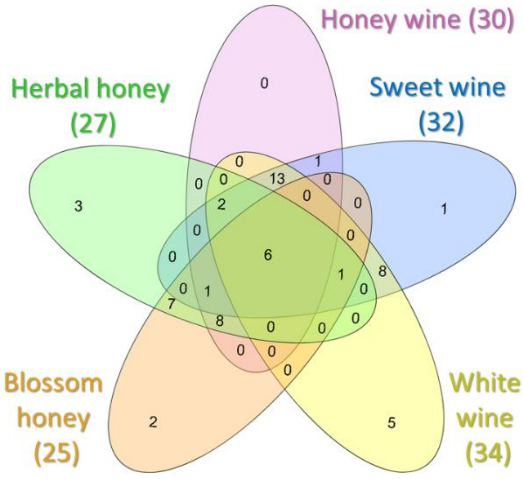
COMPARATIVE NMR SPECTROSCOPY ANALYSIS OF HONEY, WINE AND HONEY WINE (MEAD)

Dessislava Gerginova, Yavor Mitrev, Svetlana Simova

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

Honey and wine are favourite food products known worldwide for their antioxidant and anticancer properties. They contain carbohydrates, amino acids, organic acids, volatile compounds and others substances with concentrations depending mainly on the botanical and geographical origin. Mead is an alcoholic beverage produces from fermented honey that increased recently its popularity, especially in England, Germany, Slovenia, Poland, Russia, South Africa, Ethiopia, Australia, New Zealand and the USA. Literature information about mead analysis is limited with main components sugars, alcohols, organic acids, volatile compounds and minerals [1]. The composition is depending on two basic factors: the type of honey and the production process [2].

The present work aims to compare the chemical composition of two honey types (blossom and herbal), two wine types (white - Chardonnay and a sweet dessert wine - Commandaria) with a honey wine with herbs by 1D (^{1}H, ^{13}C) and 2D NMR (HSQC, J-RES) spectroscopy. Different statistical techniques (Venn diagram, PCA, PLS-DA) have been applied to establish similarities in the chemical composition of honey, wine and mead.



5-set Venn diagram [3]

Acknowledgement:

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SYNTHESIS, PHOTOPHYSICOCHEMICAL PROPERTIES AND ANTI-TUMOR ACTIVITY OF LUTETIUM COORDINATED, CARBOHYDRATE-FUNCTIONALIZED PHTHALOCYANINES AS THIRD GENERATION PHOTOSENSITIZER FOR PDT

Ivelina Eneva[1], Meliha Aliosman[1], Vanya Mantareva[1], Vasiliya Kril[2], Ani Georgieva[3], Anton Kri[3],Veselin Kussovski[4], Ivan Angelov[1]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *Sorbonne University, Paris, France*
[3] *Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4] *Institute of Microbiology „Stephan Angeloff", Bulgarian Academy of Sciences, Sofia, Bulgaria*

Coordinated with metal ion phthalocyanines (MPc) are effective photosensitizers for photodynamic therapy (PDT). In order to increase selectivity and target specificity of the photo active macrocycles to tumor cells, carbohydrate ligands (Galactopyranose or Furanose) were substituted at the periphery and nonperiphery positions of Lutetium coordinated phthalocyanines (LuPc). Obtained compounds can be referred as third-generation photosensitizers.
The present work aims synthesis of lutetium Lu(III) phthalocyanines tetra peripherally and nonperipherally functionalized with galactopyranose and furanose units and comparison of their photophysicochemical and pharmacokinetic properties with the Zn (II) functionalized phthalocyanines. All compounds generated singlet oxygen $^1O_2$, with very high value of the quantum yields. In vitro assays using the human cancer cell lines: - breast carcinoma with low and high metastatic potential (MCF-7 and MDA-MB 231) respectively; colon carcinoma (HT-29) and carcinoma of the cervix (HeLa) were tested. The cytotoxicity of investigated LuPcs towards non-tumorigenic cells has been evaluated on Balb/c mouse 3T3-clone 31 cell line.

Acknowledgments: The authors thanks to the support of the National Science Fund- project KP -06-H23/8 /2018, Bulgaria.

CHARACTERIZATION OF TUNISIAN JOJOBA OIL EXTRACTED FROM SEEDS AND CAKES UNDER DIFFERENT PROCESSES AND CONDITIONS

Firas Feki[1], Sabina Taneva[2], Svetlana Momchilova[2], Iskra Totseva[2], Yana Nikolova[2], Angel Konakchiev[2,3], Vladimir Dimitrov[2], Sami Sayadi[1]

[1] *Center of Biotechnology of Sfax, Laboratory of Environmental Bioprocesses, Sfax, Tunisia*
[2] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3] *Sofia Tech Park JSC, Sofia, Bulgaria*

Jojoba (*Simmondsia chinensis*, Simmondsiacea) originates from Southwestern North America. Now it is planted in many other deserts across the world, including Tunisia, where it is cultivated in Sidi Bouzid (Meknassi). Jojoba is the only plant which produces significant quantity of liquid wax (over 50%) in seed oil and hence it can be applied as natural emollient for all skin types and hair. The wax is stable and resistant to both oxidation and rancidity. Therefore Jojoba Oil (JO) is used as a carrier substance for sensitive components such as vitamin A [1], essential oils for aroma- and massage therapy [2], for stabilization of penicillin products [3], etc.

This study aims to the lipid analysis of Tunisian JO from seeds and cakes in order to evaluate qualitative and quantitative differences between oil samples obtained under different process and extraction conditions, namely cold pressing (at 25^{o}C), heat pressing (at 70^{o}C) and hexane (Soxhlette) oil extraction from seeds and cakes. Firstly, the profiles of neutral lipid classes, as well as compounds after hydrolysis of oil including non-saponifiable fraction, were compared using High performance thin layer chromatography on silica gel. Then data about oils yield, fatty acids composition, acid value, peroxide value, conjugated dienes and trienes, and carotenoids were acquired by different analytical methods (gas chromatography, UV-spectroscopy, titration, gravimetry).

The results revealed that quality of oil regarding its lipid composition and oxidative stability depended on the temperature of extraction process (25^{o}C vs. 70^{o}C). Also, both cakes contained significant amounts of oil (13-14%) with a little higher acid and peroxide values and more conjugated dienes and trienes, but, on the other hand, with higher amount of carotenoids.

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Acknowledgments: This work is carried out within the project EXANDAS (H2020-MSCA-RISE-2015, grant agreement No 691247). Thanks are due to Sofia Tech Park JSC for the HPTLC and GC-MS equipment used.

EXPLORING THE ELECTROCHEMICAL REDUCTION OF NITROFURANTOIN THOUGH MODEL COMPOUNDS: A COMBINED IR AND COMPUTATIONAL STUDY

S. Stoyanov[1], D. Yancheva[1], A. Popova[1], K. Anichina[2]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2] *2University of Chemical Technology and Metallurgy, Department of Organic Synthesis, 8Kliment Ohridski Blvd., 1756 Sofia, Bulgaria*

The study on nitroaromatic drugs reduction provides important insights on the mechanisms underlying their hepatotoxicity associated with bioreduction to radical anion and other hazardous intermediates in vivo [1-3].
In the present contribution the electrochemical reduction of anti-inflammatory drug nitrofurantoin, N-(5-Nitro-2-furfurylidene)-1-aminohydantoin, an antibacterial agent used to treat acute urinary tract infections, was studied by IR spectroscopy in an electrochemical IR liquid cell. It was shown that it leads to considerable decrease of the N-O and C=O stretching frequencies and increase of the C-NO2 stretching frequency. In the same time the band for δ(N-H) disappeared. The observed IR spectral changes differ significantly from those expected on the bases of one-electron reduction to nitro radical anion, and comply better with the generation of dianion radical of nitrofurantoin. Comparison with theoretically predicted spectra of possible reduction products supports the dianion radical formation.
In order to clarify the observed IR spectral changes and assign them unambiguously a particular product, a series of IR measurements on nitrofurantoin derivatives was carried out. Nitrofurantoin was converted into anion by treatment with excess of dry CD3ONa and the corresponding spectral and structural changes, accompanying the conversion were described by IR spectra in DMSO-d6. N-methyl and oxadiazole derivative of nitrofurantoin along with a N,N-dimethyl hydrazine derivative of nitrofuran, were synthesized and their electrochemical reduction was monitored by IR spectroscopy. The structural and spectral changes arising from the conversion were assisted by DFT computations.

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Представен на 11-та химична конференция с международно участие Пловдив 11-13 октомври 2018г.

NMR STUDY OF SOLITION STATE BEHAVIOUR OF FOLIC ACID

Nikolay Petkov, Yavor Mitrev

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

Folic acid (**Fig. 1**) is particularly challenging object for NMR investigations in water, due to its low solubility bellow neutral pH, pH dependent chemical shifts and existence of aggregation and tautmeric equilibria in its solubility range. Consequently, the available NMR data is sparse, consisting mostly of ^{1}H chemical shifts and, in limited cases, of ^{13}C data at high or very low pH. Moreover, the low availability of non-labile protons in the pterine fragment additionally limits the commonly used ^{1}H-based approaches for structural investigations, especially, when D_2O is used as NMR medium.

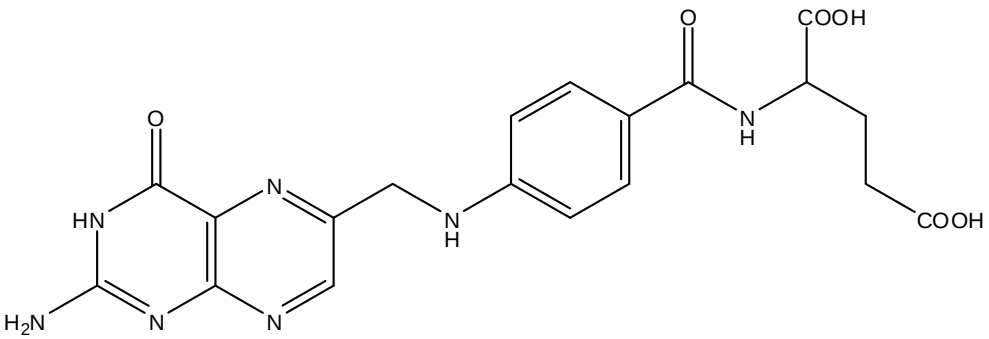

Figure 1. Structure of folic acid

Here we present our initial results from NMR investigations of folic acid in H_2O, aiming at detailed NMR characterization of its solution behaviour. Using non-deuterated solvent was found to be beneficial in this case, as it provides information for the labile NH protons, which are otherwise invisible in the ^{1}H spectra. In addition to standard ^{1}H- and ^{13}C NMR experiments at different pH, advanced techniques, e.g. for measurement of heteronuclear coupling constants (HSQMBC) and diffusion ordered spectroscopy (DOSY) were also employed and comparison between laboratory (293K) and physiological (310 K) temperature is also sought.

STUDY OF ANTIBACTERIAL ACTIVITY OF DIFFERENT MUCUS EXTRACTS OF *C. ASPERSUM* AGAINST *PROPIONIBACTERIUM ACNES*

Aleksandar Dolashki[1], Lyudmila Velkova[1], Petya Hristova[2], Pavlina Dolashka[1]
[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences*
[2] *Sofia University "St.Kliment Ohridski", Faculty of Biology, Bulgaria*

Snails produce biological fluid (mucus) which protects them against microbial invasion. The mucus of garden snail *C. aspersum* is a complex mixture of bioactive compounds with potential pharmacological application.
Propionibacterium acnes is a Gram-positive, microaerophilic and lipophilic bacterium. Recent microbiological and dermatological studies have pointed to the strong associations between *P. acnes* and acne vulgaris, *S. aureus* and atopic dermatitis, and Malasseziaspecies with dandruff. In some patients, *P. acnes* can cause severe infections, including endocarditis, intravascular infections, central nervous system infections, endophthalmitis, and, rarely, arthritis. Resistance development in *P. acnes* is another serious problem in using conventional antibiotics such as erythromycin. In these contexts, we investigated several mucus extracts from snail *C. aspersum* for antibacterial activity.
The isolated mucus extract was separated into several fractions by ultrafiltration on Millipore membrane filters from 1kDa, 10kDa, 20kDa, 50kDa and 100kDa. The obtained fractions were tested for antimicrobial activity against *P. acnes* 266.
The fractions 1-10kDa and 1-20kDa, followed by a fraction above 100kDa possess significant antibacterial activity against *P. acnes* 266. Using *de novo* sequencing (MALDI-MS/MS analysis) we identified the primary structures of above 20 novel antimicrobial peptides with molecular mass between 1-5kDa. Most of them contain high level of glycine, proline, tryptophan and valine residues which are typical for peptides with antimicrobial activity.
We have applied a combination of two-dimensional electrophoresis (2-DE) and mass spectrometry (MALDI-MS) to identity changes in protein expressions of cell extract of *P. acnes*, before and after treatment with active fraction 1-10kDa. We have detected significant decrease in protein expression of: PPA2105 (MW 35972Da), triacylglycerol lipase precursor; PPA0687 (MW 28612 Da) CAMP factor 2; PPA2127 (MW 41735Da) putative adhesion or S-layer protein; PPA2106 (MW 54063Da) putative endoglycoceramidase; PPA1939 (MW 16862Da) hypothetical protein specific to *P.acnes*. Some of these proteins are factors for the virulence of *P. acnes* and other pathogenic bacteria.
Our research may be considered as basic information about mechanism of antibacterial activity of mucus from *C. aspersum* against *P. acnes* and its potential medical application.

Acknowledgments: *This work was supported by the research project DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.*

STUDY OF AN EXPERIMENTAL ANIMAL MODEL WITH SCOPOLAMINE-INDUCED NEURODEGENERATIVE DISORDER

Lyudmila Velkova[1], Aleksandar Dolashki[1], Lyubka Tancheva[2], Liana Alova[2], Wolfgang Voelter[3], Pavlina Dolashka[1]

[1]*Institute of Organic Chemistry with Centrum of Phytochemistry, Bulgarian Academy of Sciences, G. Bonchev 9, 1113 Sofa, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofa, Bulgaria*
[3]*Interfacultary Institute of Biochemistry, University of Tübingen, Hoppe-Seyler-Strasse 4, D-72076 Tübingen, Germany*

Alzheimer's disease (AD) affects approximately 35.6 million people worldwide. It causes mental and cognitive deficits such as impaired memory, intellect and personality disorder. The pathological features of this disease include extensive extracellular deposits of β-amyloid (Aβ) plaques, intracellular neurofibrillary tangles, as well as subsequent neuronal and synaptic loss, which often begin several years prior to memory loss and the damage is already irreversible at the time of diagnosis.
In this study, brain homogenate and cerebrospinal fluid (CSF) of an experimental rat model in normal and scopolamine-induced neurodegenerative disorder (type AD), were analyzed by using proteomic techniques, mass spectrometry (MALDI-TOF) and bioinformatics' analysis.
Using two-dimensional gel electrophoresis, changes were detected in brain rat proteome before and after treatment with scopolamine. Our proteomic analysis revealed many proteins with aberrant expression: above 20 proteins have significantly decreased their expressions: dynamin, dihydropyrimidinase-related protein, heat shock cognate 71kDa protein, α and β tubulins, 14-3-3 proteins, etc. Furthermore we found that other proteins significantly increased their expression, such as: microtubule-associated protein tau, actin, α enolase, and peroxiredoxin.
Amyloid can be measured in CSF and Aβ 1-42 levels indicate abnormal amyloid plaque aggregation in the brain. The intact Aβ from soluble aggregates comperes are especially relevant biochemical marker because they are believed to be the most toxic form of Aβs. We investigated different fractions <10kDa from CSF by MALDI-MS and found a diversity of Aβ peptides below-6.5kDa, and identified some of them. Cerebrospinal fluid biochemical markers for AD would be of great value to improve the clinical diagnostic accuracy of the disorder.

Acknowledgments: *This work was supported by the Bulgarian Ministry of Education and Science under the National Research Program "BioActivMed" approved by DCM № 658/14.09.2018.*

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