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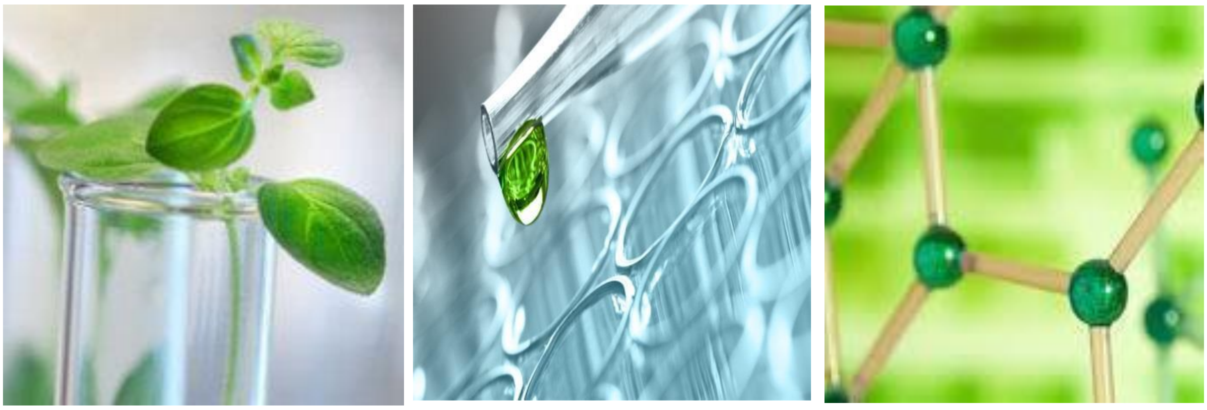
ИНСТИТУТ ПО ОРГАНИЧНА ХИМИЯ С ЦЕНТЪР ПО ФИТОХИМИЯ

15-16 НОЕМВРИ 2017 г.

ПОСТЕРНА СЕСИЯ – РЕЗЮМЕТА



COMBINED STUDIES TOWARDS THE AGGREGATION OF TRIMETHINE CYANINE DYES

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Over the past couple of decades, cyanine dyes have attracted a significant medicinal, biological and biochemical scientific interest due to their unique photophysical properties, such as their low toxicity and remarkable fluorescence enhancement upon binding to bio-objects. These favourable characteristics, along with their long-wavelength absorption / emission wavelengths and outstandingly high molar extinction coefficients (> 100.000 M^{-1} cm^{-1}), define the importance of their use as diagnostic tools for the recognition of polynucleotides. Our recent studies [1], revealed that the trimethine cyanine dyes AK3-4 and AK3-9 (Figure 1) illustrate a highly selective response to specific DNA/RNA sequences. This observation was a consequence of the dye molecules self-association into H-dimers.

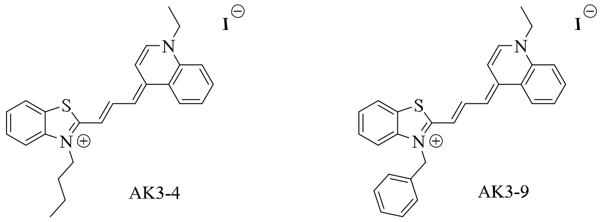

Figure 1. Chemical structures of the compounds under investigation.

Namely, the former results inspired us to shed more light into the aggregation process of the title compounds. Therefore, we employed chemometric analysis [2], crystallographic studies and quantum chemical calculations, aiming to fully profile the dimeric species, and the equilibria between the monomers and the dimers. Herein, our concept was achieved by: i) the investigation of the photophysical properties of the trimethine cyanines in aqueous media adjusting the temperature, the concentration, varying the path length and introducing a polar aprotic solvent (DMSO), ii) advanced processing of the experimental data, which provides information on the strength of the dimer assembly, iii) verification of the experimental results by obtaining x-ray crystal structure, and vi) quantum chemical calculations.

Acknowledgements:
The generous support from the Swiss National Science Foundation by the SCOPES Program (Tautocrowns Joint Research Project, SupraChem@Balkans.EU and SupraMedChem@Balkans.Net Institutional Partnership Projects) and the Bulgarian National Science Fund (access to MADARA computer cluster by project RNF01/0110) are gratefully acknowledged.

References:

1. Kurutos, A.; Crnolatac, I.; Orehovec, I.; Gadjev, N.; Piantanida, I.; Deligeorgiev, T. (2016) *J Lumin*, 174, 70-76.
2. Antonov, L; Gergov, G; Petrov, V; Kubista, M; Nygren J. (1999) *Talanta*, 49, 99-106.

NEW TOCOTRIENOLS FROM COLOMBIAN PROPOLIS

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Propolis is a bee hive product providing social immunity to the bee family. It is well known to men for its preventive and healing properties and nowadays it is included in various products available on the market. As a mixture of plant resinous exudates and beeswax, its chemical composition depends strongly on the local flora and thus on the climatic characteristics at the site of resin collection. For this reason, many and structurally diverse compounds (flavonoids, phenolic acids, terpenes, stilbenes, xanthones, etc.) are isolated and many propolis types with well known chemical profiles are formulated, most of them for propolis originating from tropical regions due to the diverse tropical flora. Thus, the dealing with propolis from unexplored/scantily explored regions has the potential to uncover new biologically active compounds with important pharmacological effects.

We studied propolis from five regions of Colombia, a country with tropical climate from where propolis is scantily investigated. Following the propolis type dereplication strategy [1] GC/MS analysis of the total extracts was applied at the first step. The chemical profile of all samples studied was similar but did not match any known propolis type. Thus, through detailed chemical analysis two new natural compounds and one new for propolis delta-tocotrienols derivatives with oxidized chromanol ring and terminal side-chain were isolated. The structures were identified by means of UV, IR, MS and NMR methods.

The isolated compounds are the first tocopherol derivatives found in propolis. Their plant origin and biological activity are subjects of further investigations.

References:

1. Bankova V, Bertelli D, Borba R, Conti BJ, da Silva Cunha IB, Danert C, Eberlin MN, Falcão SI, Isla MI, Moreno MIN, Papotti G, Popova M, Santiago KB, Salas A, Sawaya ACHF, Schwab NV, Sforcin JM, Simone-Finstrom M, Spivak M, Trusheva B, Vilas-Boas M, Wilson M, Zampini C (2016) Standard methods for Apis mellifera propolis research. Journal of Apicultural Research DOI: 10.1080/00218839.2016.1222661

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α-CYCLODEXTRIN: HOW EFFECTIVELY CAN ITS HYDROPHOBIC CAVITY BE HYDRATED?*

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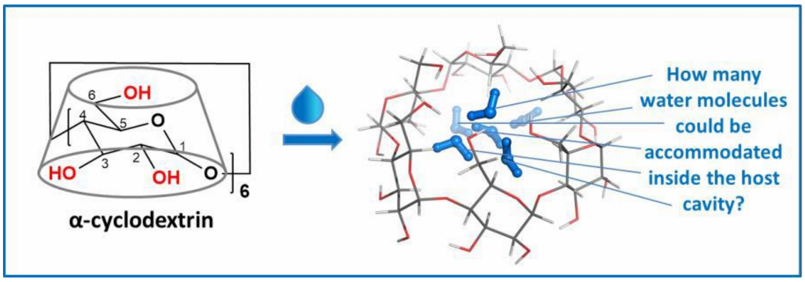
Cyclodextrins (CDs) are well-known macrocyclic molecules with distinctive "doughnut" shape and ability to form inclusion complexes with various molecular objects of interest to pharmacology, food industry, cosmetics, catalysis and environmental protection. The CD walls are hydrophobic while the two rims, decorated with OH groups, are hydrophilic. As a result, the exterior of CDs is hydrophilic whereas the cavity is hydrophobic, which gives cyclodextrins the capability to accommodate mostly hydrophobic substances inside their cavity. Supramolecular structures with CDs as host molecules exist in both the solid state and in solution. However, the internal cavity can bind hydrophilic objects as well, though with lower affinity than the respective hydrophobic moieties. In fact, the cavity of a guest-free cyclodextrins is never empty - it hosts water molecules. Several questions regarding the αCD hydration are answered in our study by combining experimental measurements (differential scanning calorimetry and thermogravimetry) with theoretical calculations (DFT computations): (1) Which spots of the αCD cavity ("hot spots") have the highest affinity for the guest water molecules? (2) How stable are water clusters inside the cavity - should the water molecules be hydrogen bonded to each other or separate binding to the cavity "hot spots" will suffice? (3) Which mode of water binding - sequential (by individual water molecules) or bulk (by pre-formed water clusters) - is thermodynamically more favored? (4) What is the upper limit of the number of water molecules bound inside the host cavity? (5) What factors do control the αCD hydration process?



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MOLECULAR INSIGHT INTO INCLUSION COMPLEX FORMATION OF CURCUMIN AND CALIX[4]ARENE*

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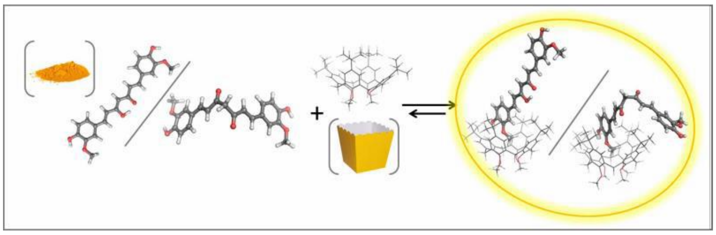
Curcumin is a natural yellow-orange pigment found in the Indian spice turmeric. It is a relatively small molecule but possesses an enormous theraupeutic potential [1]. Although curcumin has shown efficacy against numerous human ailments, its poor bioavailability limits its therapeutic efficacy. Curcumin has extremely low water solubility [2]. In addition to the poor water solubility, the design and elaboration of parenteral curcumin formulations are further limited by photodegradation processes. Encapsulation of bioactive compounds can be used both to improve bioavailability and to protect them against harm conditions. In previous study a representative PEO-modified *tert*-butylcalix[4]arene bearing four polyethylene oxide (PEO) moieties was studied as a curcumin delivery carrier. The curcumin:modified calixarene inclusion complex was further advanced to a hybrid delivery system via encapsulation in plain dipalmitoylphosphatidylcholine (DPPC):cholesterol (CHOL) liposomes [3]. The structural analysis of the obtained complexes has shown that the keto-enol tautomeric form is presented in the complex and a hypothesis for non-fitting to calix[4]arene cavity "roof-like" structure of KK form has been proposed. In the DFT study herein the effect of the complex formation with calix[4]arene on the tautomerism of curcumin was studied by means of theoretical calculations at M062X/6-31G(d,p) level of theory. The results show that both tautomeric forms can enter and leave the host cavity without sterical problems. The binding of the diketo tautomeric form was found to be more favorable (by 2.1 kcal mol^{-1} in the gas phase and 1.7 kcal mol^{-1} in water) than the binding to the same host system of the preferred keto-enol tautomeric form. This results in attenuation of the energy difference between the tautomers in the complex, but does not shift dramatically the position of the tautomeric equilibrium in the guest molecule.



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References:

1. Araujo, C. C.; Leon, L. L. (2001) Mem. Inst. Oswaldo Cruz 96, 723-728.
2. Modasiya, M. K.; Patel, V. M. (2012) Int. J. Pharm. Life Sci. 3, 1490-1497.
3. Drakalska, E.; Momekova, D.; Manolova, Y.; Budurova, D.; Momekov, G.; Genova, M., Antonov, L.; Lambov, N.; Rangelov, S. (2014) Int. J. Pharm. 472, 165-174.

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DETERMINANTS OF THE HOST-GUEST INTERACTIONS BETWEEN α-, β- AND γ-CYCLODEXTRINES AND GROUP IA, IIA AND IIIA METAL CATIONS: A DFT/PCM STUDY*

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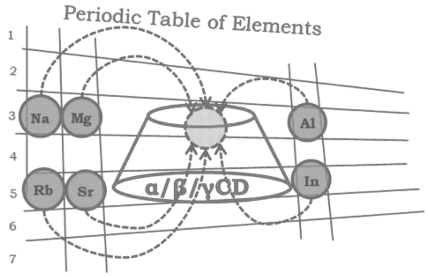
The most widely used native cyclodextrins are α-, β- and γ-cyclodextrins containing six, seven or eight α-D-glucopyranoside units in the ring, respectively. Although their ligation properties have been extensively studied, a number of questions regarding their metal binding and selectivity remain unaddressed: To what extent does the size and flexibility of the host α-, β- and γ-cyclodextrin influence its metal affinity/selectivity? Which metal is the most preferred binding partner of α-, β- and γ-cyclodextrins? How do the charge, size and coordination number of the metal cation shape its interactions with the host cyclodextrin? Can the guest metal cation inflict structural alterations in the host molecule and, if so, how do these changes correlate with the metal's properties? In the present study, by employing density functional theory (DFT) calculations combined with polarizable continuum model (PCM) computations, we answer these questions by evaluating the thermodynamic parameters of the IA, IIA and IIIA group metal binding to α, β- and γ-cyclodextrins. We assess how the interaction between the two binding partners depends on (1) the size, valence state and coordination number preference of the guest metal cation, (2) size and flexibility of the host molecule, and (3) dielectric properties of the environment. The series of group IA (Na^+ and Rb^+), IIA (Mg^{2+} and Sr^{2+}) and IIIA (Al^{3+} and In^{3+}) metal cations has been chosen for the task as it allows to study the effect of various metal parameters (variable charge, ionic radius and coordination number) on the strength and form of the interactions with the host cyclodextrins. The results obtained shed light on the intimate mechanism of the metal binding to α-, β- and γ-cyclodextrins and unveil the key descriptors of the process.



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A CONCEPT FOR STIMULATED PROTON TRANSFER IN 1-(PHENYLDIAZENYL) NAPHTHALEN-2-OLS

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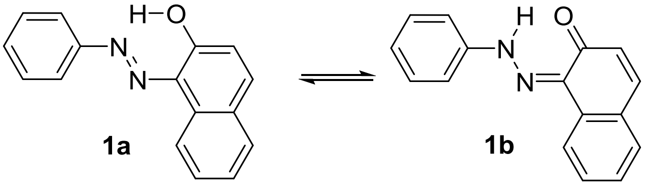
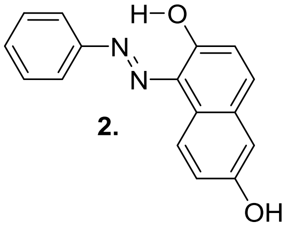


1a **1b**



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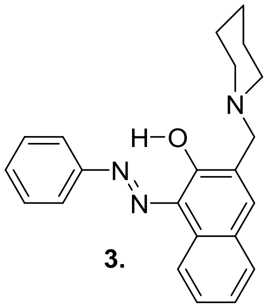

3.

A series of arylazoderivatives of naphthols (**1**-**3**) were studied by means of UV-Vis spectroscopy in different solvents as well as by quantum chemical calculations (MO6-2X/TZVP). Previous studies have shown that 1-(phenyldiazenyl)naphthalene-2-ol (commonly known as Sudan 1 (**1**) exists as a tautomeric mixture.

The effect of the solvents is minimized by the existing intramolecular hydrogen bond. In general in non-polar solvents such as i-octane and tetrachloromethane1aform predominates, whilst in more polar solvents (methanol and formamide) the opposite holds true (**1b**) [1].

The aim of the current study is to investigate how structural modifications can influence the tautomeric proton exchange. Structure **2** contains an additional OH-group which is expected to deprotonate easily and to affect the position of the tautomeric equilibrium by changing the electronic properties of the substituent (from OH to O^-).

The implementation of a sidearm in **3** is expected to create a condition for competition between nitrogen from azo group and from piperidine unit for the tautomeric proton. In analogy with already existing tautomeric switching systems, developed by us [2] the use of acid as stimuli for controlling the tautomeric process could be foreseen.

Acknowledgements: The financial support from Swiss National Science Foundation (SupraChem@Balkans.eu Institutional partnership project) as well aby Bulgarian National Science Fund (access to MADARA computer cluster by the project RNF01/0110) is gratefully acknowledged.

References:

1. L. Antonov, S. Stoyanov and T. Stoyanova (1995) *Dyes and Pigments*, **27** 133.
2. L. Antonov, V. Deneva, S. Simeonov, V. Kurteva, D. Nedeltcheva and J. Wirz (2009) *Angew.Chem., Int. Ed.*,**48**, 7875.

GLYCEROL ESTERIFICATION ON SO_3H-MODIFIED MESOPOROUS SILICAS

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The increasing demand for energy, chemicals and materials in our society has lead research activity towards the development of green technologies, based on renewable resources. Biofuels are an attractive alternative to fossil fuels due to their positive influence on the environment and the fact that they are produced from renewable sources. The production of biodisel on the basis of waste has given research a new spur due to the low cost of the initial raw material, as well as the desire to utilize waste and solve the economic, environmental and social problems. Glycerol generated as a byproduct in conventional biodiesel production can be esterified with acetic acid to obtain valuable products which have found versatile industrial applications.

In the present study SBA-15 and SBA-16 materials were modified with SO_3H groups and tested in the glycerol esterification with acetic acid.

Functionalization with SO_3H groups was done in two-step procedure, in which mercapto-modified mesoporous silica will be obtained first in reaction with mercaptosilane followed by treatment in H_2O_2 resulting in SO_3H modified mesoporous silicas. The initial and the SO_3H-containing mesoporous materials were prepared by post synthesis method and characterized by X-ray diffraction (XRD), N_2 physisorption, TPD of ammonia and solid state ^1H NMR spectroscopy. The catalytic performance of SO_3H/SBA-15 and SO_3H/SBA-16 is studied in glycerol esterification at different reaction temperatures. Sulfonic-functionalized SBA-15 mesoporous material shows total conversion of glycerol and about 80 % selectivity to di- and triacetylglycerols at 130 ^oC reaction temperature.

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COMPARATIVE STUDY OF ALKALOID PROFILE OF THREE *HYPECOUM* SPECIES

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The genus *Hypecoum* L. (Papaveraceae) is represented by 18 species growing in the Mediterranean region, Central Asia and China. The plants are used in Tibetan medicine as an antipyretic, analgesic and anti-inflammatory remedy [1]. The plants are known for their biologically and pharmacologically active isoquinoline alkaloids, such as protopines, protoberberines, aporphines, benzophenantridines, spirobenzylisoquinolines and secoberbines [2].

Alkaloid profiles of *Hypecoum pendulum* L. (Algeria), *Hypecoum procumbens* L. (Bulgaria) and *Hypecoum ponticum* Velen. (Bulgaria) - an endemic species for the Balkan region, were investigated and 10 isoquinoline alkaloids were determined. The main alkaloid of tertiary alkaloid mixtures in all analyzed samples were protopine. From quaternary alkaloid mixtures of *Hypecoum procumbens* and *Hypecoum ponticum* were identified the alkaloids N-methilcanadine and N-methylstylopine. These alkaloids were found for the first time from the species *Hypecoum ponticum*.

One new natural alkaloid with quaternary structure was isolated from *Hypecoum ponticum* and its structure was determined on the basis of detailed spectroscopic analysis, including1D and 2D NMR and EI MS.

References:

1. Willis J. C (1966) A Dictionary of the Flowering Plants and Ferns (7th ed.) Cambridge University Press, Cambridge, pp. 569.
2. Preininger V (1986) Chemotaxonomy of Papaveraceae and Fumariaceae. In: Brossi, A. (Ed.), The Alkaloids, vol. 29. Academic Press, London, pp. 1–98.

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NEW SESQUITERPENE LACTONES FROM *INULA OCULUS-CHRISTI* L.

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Genus *Inula* (Asteraceae) contains more than one hundred species widespread mainly in Africa, Asia and Europe. Sixteen *Inula* species are known as plants used in traditional medicine and some of them are constituents of medicinal herbal preparations. Plants belonging to this genus have shown to possess various biological activities, attributed to the presence of sesquiterpene lactones, phenolic acids, and flavonoids [1].

Continuing our research on *Inula* species, growing in Bulgaria we have focused our attention on sesquiterpene lactones in *I. oculus-christi*. The aerial parts were extracted with chloroform. Further CC (Sephadex LH-20 and Silica gel) and PTLC purification of the extract afforded 9 sesquiterpene lactones – the new compounds 9β,10β-epoxy-gaillardin, 9α,10α-epoxy-2-*epi*-gaillardin, 2α-acetoxy-4α,9β-dihydroxy-1β-guaia-11(13),10(14)-dien-12,8α-olide, 9α-hydroxyinuchinenolide B, 9β-hydroxy-2-*epi*-inuchinenolide B and 4α,15α-epoxypulchellin E, in addition to known gaillardin, pulchellin C, pulchellin E. The structures and relative stereochemistry were determined by 1D and 2D NMR spectroscopy (^{1}H, ^{13}C, COSY, HSQC, HMBC and NOESY) and MS. The isolated compounds were with guaiane and eudesmane carbon skeleton. It was found that gaillardin was the principal one like in *I. oculus-christi* of other origins [2, 3]. Pulchellin C was detected in the plant from Iran [2], while pulchellin E – in *I. oculus-christi* from Montenegro [3].

Our results are consistent with the previous reports and enrich the knowledge on the lactone profile of *I. oculus-christi* with the newly identified components. The obtained results could be of chemotaxonomic interest.

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

References

1. Seca, A.M.L., Pinto, C.G.A., and Silva, A.M.S. (2015) Metabolomic Profile of the Genus *Inula*. Chem. & Biodiv. 12: 859-906.
2. Mosaddegh, M., Moghadam, M.H., Ghafaria, S., Naghibi, F., Ostad, N.S., Read, R.W. (2010) Sesquiterpene lactones from *Inula oculus-christi*. Nat. Prod. Commun. 5: 511-514.
3. Vajs, V., Nevescanin, M., Macura, S., Juranić, N., Menković, N., MilosavlJević, S. (2003) Sesquiterpene lactones from the aerial parts of *Inula oculus-christi*. Fitoterapia 74: 508–510.

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CHEMICAL PROFILE AND ANTI-LIPASE PROPERTIES O*F JURINEA TZAR-FERDINAND II* DAVIDOV

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Jurinea tzar-ferdinandii Davidov (genus *Jurinea*, Asteraceae family) is a Balkan endemic plant with limited distribution in Bulgaria and Romania. The literature survey did not show any reports concerning its chemical constituents and biological activity.

The aim of this study was to investigate chemical constituents of this species and to evaluate their anti-lipase properties. The aerial parts of *J. tzar-ferdinandii* were extracted with chloroform. CC and PTLC of the resulting chloroform extract led to isolation and identification of 26 compounds, belonging to 3 main groups of secondary metabolites: triterpenoids (α- and β- amyrin, lupeol, taraxasterol and ψ-taraxasterol, and the corresponding 3-O-acetates and 3-O-palmitates), sesquiterpene lactones (onopordopicrin and 5 biogenetically related germacranolides, eudesmanolides and an elemanolide) and flavonoids (pectolinarigenin, eupatorin and jaceosidin). Their structures were elucidated using spectral methods (NMR, MS, UV and IR).

Inhibitory activities of the fractions containing triterpene alcohols, triterpenyl acetates, triterpenyl palmitates, sesquiterpene alcohols and flavonoids against a bacterial lipase from *Candida rugosa* (CRL) and a lipase from porcine pancreas (PPL) were assessed in a spectrophotometric assay. The estimated half maximal inhibitory constants (IC_{50}) for the two enzymes were in the range of 70-150 µg/mL. The strongest inhibitory activity was found for the fraction of the triterpenyl palmitates, and the estimated IC_{50} values were 80.1±2.4 µg/mL and 85.4±3.5µg/mL for CRL and PPL, respectively. The results are comparable with those reported in the literature for the tetrahydrolypstatin, an irreversible inhibitor of lipases from Candida sp. and human pancreatic lipase [1].

References

1. Khedidja B., Abderrahman L. (2011) Selection of orlistat as a potential inhibitor for lipase from *Candida* species, Bioinformation 7: 125–129.

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DEVELOPMENT OF NEW NANOCOMPOSITES FOR CONTROLLED DELIVERY OF ANTI-INFLAMMATORY AND ANTINEOPLASTIC DRUGS

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Mesoporous silica materials attracted interest as a carriers in drug delivery systems because of their unique properties such as tunable pore size, particle size and morphology, large pore volume, high specific surface area (>700 m^2/g), good chemical and thermal stability and dual-functional surface (external and internal) [1]. Some problems of bioactive molecules as low solubility, poor bioavailability, degradation before reaching the target, toxic side effects etc. can be solved by their loading in composite mesoporous materials. Developing of magnetic-silica and polymer-silica nanocomposites give a new push in stimuli responsive supply of the drug to the targeted organ or tissue of the body.

In the present project we developed systems for dermal delivery of quercetin, oral delivery of prodrug sulfasalazine and intraperitoneal delivery of prednisolone, based on modified mesoporous nanocomposites.

Delivery systems for quercetin on the basis of parent and modified with cations of transition metals (Ag, Zn) mesoporous silica materials type MCM-41, SBA-16 and SBA-15 were developed. High capacity loading (over 40%) of quercetin molecules into the channels of the materials was achieved by incipient wetness impregnation method. For the first time the formation of stable Zn-quercetin complex loaded on mesoporous silica materials was evidenced. *In vitro* experiments at pH=5.5 showed faster quercetin release from non-modified in comparison to Zn-modified systems. The comparative cytotoxic experiments show that quercetin encapsulated in Zn-modified silica carriers proved to exert superior antineoplastic potential against HUT-29 cells compared to free drug.

Anti-inflammatory prodrug sulfasalazine, used for treatment of gastrointestinal diseases, was successfully loaded in amino-modified MCM-41 and SBA-15 spherical nanoparticles by incipient wetness impregnation method. As prepared systems was coated with polyelectrolyte complex of polymers with pH- and time-responsive solubility. *In vitro* release showed controlled release of the drug in the target pH=7 and no release at pH=1.2. The developed systems are an efficient platform for controlled delivery of prodrug sulfasalazine.

New composites type magnetic mesoporous silica (MMS) were synthesized by variation of the experiment conditions (pH, temperature, ratio of the precursors). The obtained nanocomposites are core/shell type with spherical shape, size 100 nm and high specific surface area (>800 m^2/g). MMS were loaded with prednisolone. *In vitro* release at pH=7 showed controlled prednisolone release. The *in vivo* experiments show that prednisolone encapsulation into MMS did not compromise its intrinsic pharmacological activity.

Acknowledgements: The study was financially supported by ДФНП-191/14.05.2016 project.

References:

1. Colilla M., Vallet-Regi M., *Comprehensive Biomaterials* **4** (2011) 497–514.

EXPLOITATION OF MASTIC GUM PROCESSING BY-PRODUCT FOR THE RECOVERY OF BIOACTIVE COMPOUNDS

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Mastic gum, the resin of *Pistacia lentiscus* L., has been used in traditional Greek medicine for various gastrointestinal disorders for over 2,500 years. The resin is a valuable market product itself but it is also used to produce another important product: mastic essential oil, which is widely used in the fields of pharmaceuticals, food and cosmetics. After distillation of the essential oil, a by-product remains, which contains a lot of bioactive substances, but till now it has only been used in some varnishes. In order to reveal the health promoting potential of the by-product, we performed chemical profiling using GC-MS and ^{1}H-NMR spectra and compared the profiles with the ones of the native mastic gum. The profiles were very similar, with only small quantitative differences. In both cases, the major constituents were moronic acid, masticadienonic and isomasticadienonic acid, oleanonic acid and oleanonic aldehyde. These compounds are known to possess valuable biological properties: moronic acid is active against HIV and Herpes virus, masticadienonic acid and oleanonic acid have demonstrated significant activity against *Helicobacter pylori*. These results demonstrate that the by-product of mastiha essential oil production has a great potential as a source of important biologically active compounds.

Acknowledgements: Financial and technical support from the EXANDAS project (H2020-MSCA-RISE-2015, Grant Agreement No 691247)

References:

1. Yu D, Sakurai Y, Chen CH, Chang FR, Huang L, Kashiwada Y, Lee KH. (2006). Anti-AIDS agents 69. Moronic acid and other triterpene derivatives as novel potent anti-HIV agents. *Journal of medicinal chemistry,* 49: 5462-5469
2. Paraschos S, Magiatis P, Mitakou S, Petraki K, Kalliaropoulos A, Maragkoudakis P, Mentis A, Sgouras D, Skaltsounis A-L. (2007) In vitro and in vivo activities of Chios mastic gum extracts and constituents against *Helicobacter pylori*. *Antimicrob. Agents Chemother.* 51: 551 - 559

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CHEMICAL CONSTITUENTS OF CYTOTOXIC EXTRACT OF PROPOLIS FROM PITCAIRN ISLAND

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Propolis (bee glue) is a resinous hive product collected by honeybees from certain plant sources. It has a long history of being used in traditional medicine and nowadays it is extensively used in food to improve health and prevent diseases such as inflammation, heart disease, diabetes, and even cancer. Because of its broad spectrum of biological activities there is a renewed interest in the composition of propolis, which depends on the vegetation of the area from which propolis was collected. There are numerous reports in the literature on the isolation and structural elucidation of biologically active phytochemicals from propolis collected in Europe, South America, Asia and the Pacific region. However there is no data about chemical composition and biological activity of propolis from Pitcairn Islands. In the last years it is of growing commercial interest, due to Pitcairn's bee population is disease free and their products are considered as clean of pollutants.

In our continued work on propolis of various origin we found that dichloromethane extract of propolis collected from Pitcairn Island possess *in vitro* cytotoxic activity towards triple-negative breast cancer cell line MDA-MB-231. The results showed that this extract inhibits the growth of the MDA-MB-231 line in a dose-dependent and time-dependent manner. Upon the cell growth inhibition propolis extract provokes apoptotic changes in cell nuclei. Since no previous research has been reported on it, we carried out a detailed chemical investigation. The work led to the isolation of 4 new cycloartane type triterpenes, together with 10 known individual compounds (di- and triterpenes) and five inseparable mixtures (triterpenes and phenolic lipids). Their structures were elucidated by extensive analysis of spectroscopic data (1D and 2D NMR, IR, HREIMS) and comparison with previously published reports. These findings certify the potential role of propolis in prevention and therapy of cancer and will strengthen the propolis implementation in different healthy products.

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ASSESSMENT OF POTENTIAL ORGANIC POLLUTANTS IN LEACHATES FROM BULGARIAN LIGNITES

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In this study, lignites from Thrace- and Sofia- coal basins, i.e. “Maritza-East” and “Stanjanci” mines, were subjected to water sequential extraction. The protocol of extraction was partly adopted from Doskočil et al. [1]. The process was tracked by leachate pH and conductivity. The freeze-dried extracts (leachates) were characterized by yield, technical and elemental analysis. Leachates were characterized by a set of analytical techniques, i.e. X-ray photoelectron spectroscopy (XPS) and Infrared spectroscopy (IR).

Chemical structural assignments of each component were done by curve-fitting method of the XPS, taking into account the binding energies reported for C-, N- and S-functional groups. The main surface species include carbon atoms in aromatic and aliphatic structures in Maritza and Stanjanci leachates and their contribution is 48-53 atomic % from the total C 1s signal. In samples under consideration the most intensive N 1s signal is for pyrroles and amines at 400 eV (1-3 at. %). The most abundant sulphur form is inorganic sulphates while the other ones are less than 1%. In this case the Maritza sample sulphatic sulphur content (13.6%) is almost seven times higher than the same for Stanjanci leachate. The FTIR spectra of the fractions show mainly the presence of oxygen-containing functional groups.

The analysis at molecular level showed that the leachates contain organic compounds such as benzene carboxylic acids and their derivatives, short-chain aliphatic diacids, fatty acids and polyols.

At the moment, it seems that the identified compounds do not represent an acute toxic risk from an environmental viewpoint. However, N-containing compounds could raise concerns and further attention is needed to be focused on them [2]. In progress, the study could be developed by the appraisal of the influence of different extractants, i.e. acidic leaching liquor, alkaline leaching solution and other experimental parameters (liquor: solid ratio, pH, temperature, time, etc.) on the yields and compositions of leachates.

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References:

1. Doskočil, L., Grasset, L., Enev, V., Kalina, L., Pekar, M., (2014) Study of water-extractable fractions from South Moravian lignite, *Environ. Earth Sci.,* vol. 73, pp. 3873–3885.
2. Finkelman, R.B., Orem, W.H., Castranova, V., Tatu, C.A., Belkin, H.E., Zheng, B., Lerch, H.E., Maharaj, S.V., Bates, A.L., (2002) Health impacts of coal and coal use: possible solutions, *Int. J. Coal Geol.* vol.50, pp.425–443.

LIPID COMPOSITION OF THE TWO MOST VALUABLE MOROCCAN SEED OILS – ARGAN (*ARGANIA SPINOSA* L.) AND CACTUS *OPUNTIA FICUS-INDICA* (L.)

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The argan tree (*Argania spinosa* L., Sapotaceae) and the cactus *Opuntia ficus-indica* L. (Cactaceae) are typical plants for the semiarid regions of Morocco, with vital ecological and socioeconomic significance. For centuries their fruits kernels have been processed to obtain valuable seed oils used as remedy, as food and recently mainly for cosmetics. Nowadays, the argan and cactus oils, especially the cold-pressed ones, are among the most expensive oils in the world. A part of their production still is obtained in a traditional way by Berber women through manual separation, cleaning and grinding of nuts in stone mills.

The objects of our investigation were two argan and two cactus oils produced by cold pressing and by solvent extraction. Their lipid composition was analyzed and compared in respect to the neutral lipid classes, fatty acids and tocopherols. Oxidative stability of the four oils was evaluated as well.

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CHEMICAL COMPOSITION AND QUALITY OF THE ROSE HIP SEED OIL OBTAINED BY SUPER-CRITICAL CO_2 EXTRACTION

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Rose hip oil is obtained from seeds of Rosa canina L. fruits. It is one of the few vegetable oils with high content of the essential omega-3 18:3 (linolenic) fatty acid – above 20%. Along with it, Rose hip oil contains more than 50% of other essential fatty acid – omega-6 18:2 (linoleic). Also, the oil features by significant amounts of antioxidants of which the most abundant are tocopherols (vitamin E, mainly gamma- and alpha-tocopherol) – over 1200 mg/kg. Furthermore, Rose hip oil contains carotenoids (provitamin A, mainly beta-carotene) and other effective antioxidants. The practice has proven its successful use as effective cosmetic for nourishing and regeneration of the skin and for its protection from harmful effects of environmental pollutants. Moreover, Rose hip oil is used as dietary supplement ensuring required intake of essential fatty acids and bioactive components with pronounced beneficial effects on the cardiovascular, nervous and immune systems.

The aim of this work was to evaluate the composition and quality of Rose hip oil obtained from seeds by super-critical CO2 extraction under various conditions. For this purpose, basic oil features as fatty acid composition, tocopherols and carotenoids contents, as well as its oxidative stability, were investigated.

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PENTACHLOROPHENOL ADSORPTION FROM WATER SOLUTION BY ACTIVATED CARBON FROM POLYMER BY-PRODUCTS

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Nanoporous carbon was synthesized from polyolefin waste - polyolefin wax, a by-product from industrial production of polyethylene at low pressure. The adsorbent surface characterization demonstrate well-developed surface area and presence of surface functional groups. The obtained activated carbon was applied for adsorption of pentachlorophenol from aqueous solution. Batch adsorbing experiments were performed under different conditions, i.e. concentration of pentachlorophenol, pH, contact time, etc. The adsorption of pentachlorophenol was found to follow Langmuir equation, as well as Freundlich equation.

The results show that polyolefin wax is a suitable precursor for production of inexpensive nanoporous activated carbon for waste water purification.

Keywords: Adsorption, activated carbon, pentachlorophenol, water purification, polymer waste

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ROTARY SWITCHES AS A PERSPECTIVE PLATFORM FOR DEVELOPMENT OF MOLECULAR MOTORS

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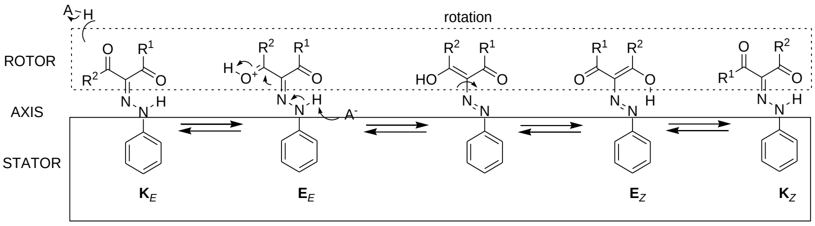
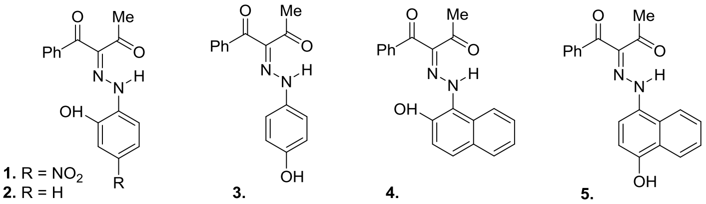
Rotor switches (molecules, containing defined parts: rotor - axis - stator) are one of possible perspective platform for development of molecular motors. Their action is based on intramolecular, acid - catalysed, proton transfer [1]. 1,2,3-tricarbonyl-2-arylhydrazones are a typical example for a molecular rotor, which exists in solution as an equilibrated mixture of intramolecularly hydrogen bonded E/Z isomers [2].



The main problem in this system is the inability to provide conditions to obtain pure isomeric forms in solution (the main requirement in the design of molecular devices).



1. R = NO_2
2. R = H
3.
4.
5.

For this reason the aim of current investigation is to design molecular rotor containing one additional tautomeric functionality (OH group) in the stator to trace how it will affect the rotation.

The possible tautomeric forms and the effects of the solvents and acidity have been investigated theoretically and experimentally.

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References:

1. Hristova S., Kamounah F. S., Molla N., Hansen P. E., Nedeltcheva D., Antonov L. (2017) Dyes Pigm., 144, 249;
2. Xin Su and Aprahamian I. (2014) Chem. Soc. Rev., 43, 1963.

IR SPECTRAL CHARACTERIZATION OF ASPIRIN PROTECT BY MEANS OF ATR AND KBR TECHNIQUE

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Research focuses on a Bayer commercial product – Aspirin Protect (AP) tablets, containing as active substance acetylsalicylic acid and other ingredients, such as: cellulose powder, maize starch, methacrylic acid-ethyl acrylate copolymer, talc and triethyl citrate. Some excepients are used as fillers, whereas others pertain to tablet coating, which is favourable to duodenum absorption.

The current study is aimed at establishing a fast and reliable method of quality control of AP main components implementing KBr or ATR techniques **(**based on complete interior reflection). Infrared spectra were recorded on *Bruker Tensor 27* FTIR Spectrometer by means of above mentioned spectral techniques. As a result of tablet additives low weight concentration, their relatively low intensity absorptions bands cannot be clearly identified in the process of KBr method implementation, which leads to insignificant difference observation within IR spectra of pure acetylsalicylic acid and ground AP.

That is why this method has proved not enough informative with respect to above mentioned components quality characterization. Due to ATR technique implementation all components contained in AP coating have been reliably determined by means of experimental IR spectra and spectral data base, used for their comparison with pure substances spectra of some excepients.

In conclusion, ATR technique has turned out to be a more reliable method of fast quality characterization of Aspirin Protect coating components.

This poster was presented at a Student Scientific Session at Faculty of Pharmacy, Medical university- Sofia on 4th November 2017.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SEVERAL MUCUS EXTRACTS FROM GARDEN SNAIL *CORNU ASPERSUM*

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Natural compounds have a great potential for use in new antimicrobial drugs, as many of them have a pronounced cytotoxicity to a number of multi-drug resistant bacteria. Snails excrete biological fluid (mucus) that has been recognized as a rich source of bioactive natural compounds and also protects them against microbial invasion.

We have investigated activities of different mucus extracts from the garden snail *Cornu aspersum* against the pathogen Gram-negative bacterial strain - *Escherichia coli* NBIMCC 878 and Gram-positive *Propionibacterium acnes,* as well as antifungal activity (*Aspergillus fumigatus* and *Aspergillus niger*).

The isolated mucus extract was separated into several fractions by ultrafiltration on Millipore membrane filters from 10kDa, 20kDa, 30kDa and 100 kDa. The obtained fractions were tested for antimicrobial and antifungal activity by the well diffusion method. We have applied MALDI-TOF/TOF mass spectrometry to determine molecule masses of compounds in active fractions. *De novo* sequencing of the peptides was performed by MS/MS analysis.

Our preliminary results have shown that the fraction below 10 kDa demonstrated strong antibacterial activity against the pathogen *E. coli*. The fraction having < 20 kDa displayed the highest antibacterial activity against *P. acnes* followed by that of >100 kDa. However, a slight activity was demonstrated by the fractions between 10-30 kDa against *E. coli*. The fraction below 10 kDa possess the most significant fungistatic activity on the mycelium growth of *A. niger* strain compared to other fractions below 20 kDa, between 30-100 kDa and above 100 kDa.

Using tandem mass spectrometry we identified the primary structures of nine novel antimicrobial peptides with molecular masses between 1-3 kDa in active fraction below 10 kDa. Most of them contain high level of glycine and leucine residues into the amino acid sequences and belong of a new class of Gly/Leu-rich AMPs. The *de novo* sequence of several peptides in the active fraction <20 kDa, revealed that most of them contain glycine, proline, tryptophan and valine which are typical for peptides with antimicrobial activity.

Our results may be considered as basic information for further investigations on bioactive peptides from *C. aspersum* and their potential biomedical applications.

Keywords: mucus extracts, garden snail *Cornu aspersum*, antibacterial and antifungal activity, antimicrobial peptides (AMPs), mass spectrometry.

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MODIFICATION WITH SODIUM PERIODATE INCREASES THE STRUCTURAL STABILITY OF HEMOCYANIN

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Hemocyanins (Hcs) are oligomeric copper-containing glycoproteins that function as oxygen carriers in the hemolymph of several molluscs and arthropods. The huge molecular size (4 to 8 MDa) of molluscan Hcs, their xenogenic character and carbohydrate content have been implicated in inducing strong immune response in mammals, which has led to the biomedical and therapeutic application of these proteins. The structural stabilization of proteins having therapeutic application is an important task. The aim of the present study is to enhance the structural stability of representatives of Hcs from molluscan species by chemical oxidation of their carbohydrate moieties.

Molluscan Hcs, used in this study, were purified by ultracentrifugation of the hemolymph extracted from marine snails *Rapana thomasiana* (RtH) and garden snails *Helix aspersa maxima* (HaH), and subsequent gel filtration chromatography and ion exchange chromatography. Carbohydrate content of RtH is 2.6% (w/w) and besides the commonly occurring sugars D-mannose, D-galactose, L-fucose, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine, this Hc contains also D-xylose and 3-O-methyl-D-galactose, unusual carbohydrates for animal glycoproteins. Carbohydrate content of 2.7 ± 0.5 % (w/w) of HaH was obtained using the phenol-sulfuric acid test. Gas-chromatographic analysis showed the monosaccharide composition of HaH is similar to that one obtained for RtH. To stabilize the structure of investigated Hcs, their carbohydrates were oxidized with sodium periodate to generate Schiff bases between the free amines and the reactive aldehydes, formed by the oxidation procedure. SDS-PAGE analysis showed differences in the mobility pattern between the native and the periodate-treated Hcs. Trypsin, which is highly selective for positive amino acids such as Arg and Lys, was not able to digest oxidized Hcs. Native and modified Hcs were equally degraded by proteinase K, an enzyme that preferentially cleaves peptide bonds adjacent to the carboxyl groups of aliphatic and aromatic amino acids, confirming the formation of Schiff bases. Local conformational changes in modified Hcs were observed by absorption and fluorescence spectroscopy although their quaternary structures were not affected. Study by microcalorimetry demonstrated an increased thermal stability of modified proteins, which may be attributed to internal cross-linking within Hc molecules as a result of the periodate treatment.

This study reveals that the oxidation of carbohydrate moiety in Hc molecules with sodium periodate leads to enhance of the structural and conformational stability of these oxygen-transport proteins.

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THERMAL STABILITY OF *HELIX ASPERSA* MAXIMA HEMOCYANIN

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Hemocyanins (Hcs) are complex oxygen-transport proteins found in the hemolymph of many arthropods and molluscs. Apart from their important biological function, Hcs are useful as immunomodulators. The thermal stability is an important characteristic of proteins having potential therapeutic applications. In the present study, the thermal unfolding of the Hc, isolated from garden snails *Helix aspersa maxima* (HaH), has been investigated by differential scanning calorimetry and UV-Vis absorption spectroscopy.

The thermal denaturation of HaH is an irreversible process. One transition, with an apparent transition temperature (T_m) at 79.8 ^{o}C, was detected in the thermogram of HaH in 20 mM HEPES buffer, containing 0.1 M NaCl, 5 mM $CaCl_2$ and 5 mM $MgCl_2$, pH 7.2, using a heating rate of 1.0 K min^{-1}. The scan rate dependence of the calorimetric profiles indicates that the thermal unfolding of the investigated Hc is kinetically controlled. The T_m and specific enthalpy values (ΔH_{cal}) for the thermal denaturation of HaH were found to be independent of the protein concentration, indicating that the dissociation of the Hc into subunits does not take place before the rate-determining step of the process of thermal unfolding started. The thermal denaturation of HaH was described by the two-state irreversible model. On the basis of this model, the parameters of the Arrhenius equation were calculated. By UV-Vis absorption spectroscopy a reversible change in the tertiary structure of the Hc, affecting the activity of the copper active sites, was found to precede the irreversible unfolding of the protein.

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EFFECT OF PHASE COMPOSITION ON THE FORMATION OF COPPER- TITANIUM-CERIUM MIXED OXIDE CATALYSTS

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Transition metal oxides are considered as appropriate alternative of noble metals and recently the main efforts are focused on the improvement of their electronic, surface and texture properties by the preparation of nanosized multi-component materials with well developed porous structure. Among them, titanium oxide has received much attention in many technological areas. The possibilities for isomorphic substitution within the crystal lattice of TiO_2 leading to the formation of an interface layer or solid solution can significantly affect not only the dispersion and structure of the obtained materials, but also could result in the creation of various defects and reduces the band gap energy and thus significantly change its redox and acid-basic properties. A lot of data in the literature demonstrates that the modification of TiO_2-CeO_2 mixed oxides with CuO increases the catalytic activity in oxidation processes due to improved dispersion of the supported nanoparticles and the textural characteristics of the composite materials. It is known that factors such as the chemical nature of the support, the acid-base functionality, and the location of the copper particles in mesoporous oxide matrix significantly affect the catalytic properties of the composite material.

The aim of current investigation is to study the effect of copper modification of mesoporous titania-ceria oxides on their catalytic behaviour in methanol decomposition and ethyl acetate oxidation. The effect of support composition on the state of hosted in it copper species is also in the focus of the investigation. Mesoporous mixed oxides were synthesized by template assisted hydrothermal technique using CTAB as structure directed agent. Copper modifications were prepared by incipient wetness impregnation of mesoporous Ti-Ce materials with aqueous solution of copper nitrate precursor and calcination in air at 773 K. The copper content in all materials was about 9 wt.%. The obtained materials were studied by a complex of physicochemical techniques such as XRD, N_2 physisorption, XRD, UV-Vis, Raman and FTIR spectroscopies and TPR of H_2. The catalytic properties of the obtained materials were studied in oxidation of ethyl acetate and methanol decomposition.

All ceria-titania materials exhibited well-developed mesoporous structure. The strong interaction between CeO_2 and TiO_2 as well as the crystallization of particles in the nanoscale range improve the redox properties of the TiO_2–CeO_2 mixed oxides. XRD data for copper modifications revealed formation of finely dispersed CuO nanoparticles with average crystallite size of 14–45 nm and this was also confirmed by Raman and UV-Vis spectra. TPR results clearly demonstrated the existence of interaction between different metal oxide species which results in the presence of more readily reducible and more finely dispersed copper oxide species. Small additives of copper to ceria and/or titania oxides promoted their catalytic activity in total oxidation of ethyl acetate and methanol decomposition to syngas, but this effect was strongly influenced by the reaction medium. The state of copper species could be successfully controlled by the support composition.

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SYNTHESIS OF NOVEL POLIDENTATE LIGANDS FOR METAL IONS' EXTRACTION

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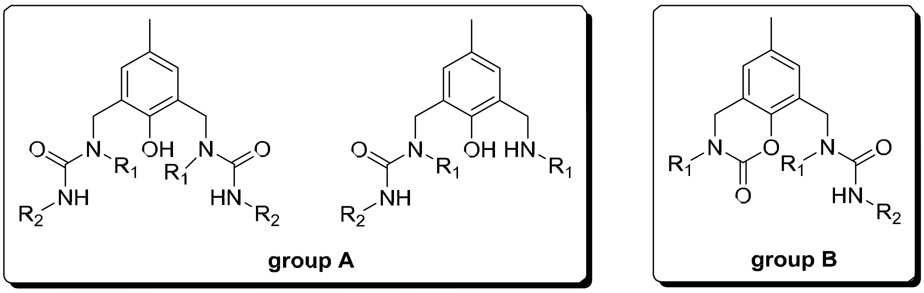
The aim of the current project is the synthesis and coordination properties study of novel polydentate ligands, combining in a common molecule the two key features of a synergistic extraction, neutral donor and chelating function. As particular objects, compounds possessing unsymmetrical NH-containing urea fragments attached to a p-cresol scaffold are chosen. The ligands can be generally divided in two groups: open-chain substituted aromatics with at least one unsymmetrical urea unit (group A) and fused aryloxazinones with unsymmetrical urea fragment (group B). The concept is to design polydentate ligands with variable coordination abilities controlled by the difference in the molecular geometry.



R_1,R_2 = aryl, benzyl, alkyl

The compounds are obtained by a fast and simple protocols from *p*-cresol-based symmetrical bis-amine, phosgene, and primary amine. Aryl, benzyl and alkyl amines are selected for both reagents (R_1 and R_2) in order to tune the nitrogen atoms' properties. The conditions are optimized by varying the reaction sequence, reagents' proportion, concentration, base, and solvent, and a series of derivatives from each group are prepared in moderate to good overall yields. The structures of the products are assigned by 1D and 2D NMR spectra and confirmed by single crystal XRD of selected samples. It is found that the open-chain substituted compounds (group A) are oriented towards optimal intramolecular H-bonding of the ureas' heteroatoms, while the preferred geometry of oxazinones (group B) is driven by intramolecular bonding. It is shown that the ligands of group A possess different geometry depending on the substitution pattern leading to different distance between the coordination centres.

The synergistic efficiency of selected examples in the isolation and separation of light lantanoids with 4-acylpyrazolone as a chelating agent and the ionic liquid N-benzyl-N'-methylimidazolium triflamide (bmimTf$_2$N) as an organic phase is studied. It is found that the ligands from groups A and B possess opposite properties; negative or positive synergism in respect to particular ions and improved separation of different ionic couples.

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EFFECT OF GROWTH REGULATORS AND PHOTOPERIOD ON ENDOGENOUS PHYTOHORMONAL LEVELS AND POLYPHENOLIC PRODUCTION IN *ARTEMISA ALBA* CELL AGGREGATE CULTURES

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Cell aggregate lines of *Artemisia alba* were selected and developed in liquid cultures: ER_3 [0.1 mg/l N^6-benzyladenine (BA) + 1.5 mg/l indole-3-butyric acid (IBA)] and ER_3_NAA [0.1 mg/l BA + 1.5 mg/l naphtylacetic acid (NAA)], grown in the dark, gyratory shaker, 100 rpm; ER_3_hν and ER_3_NAA_hν were grown in the same conditions, but at 16/8 h photoperiod. Spectrophotometric assay of the total phenolic and flavonoid compounds was performed and the samples were also tested by HPTLC analysis with chlorogenic acid, as well as 1,3-, 1,5-, 3,5-, 3,4- and 4,5-dicaffeoylquinic acids, scopoletin and fraxidin-8-glucoside as reference compounds. The analysis showed general similarity in the tested samples. Photoperiod, however seemed to have an influence on glucosylation of the samples. While dark grown suspensions exhibited biosynthesis of both the aglycons (scopoletin and fraxidin) as well as their respective glucosides (scopolin and fraxidin glucoside), the 16/8 photoperiod suspensions produced predominantly the glucosylated derivatives. These data were confirmed also by the UHPLC analysis. 3,5-dicaffeoylquinic acid was the predominant component in all samples. In terms of endogenous cytokinin production, a significant drop of their levels was observed in suspensions, where NAA represented the exogenously added auxin. The latter samples were also characterized by a significant stimulation of polyphenolics, comparable to the those produced by the differentiated shoot cultures of the plant. The obtained results are of practical interest for the targeted biotechnological delivery of scopoletin and fraxidin, which represent a higher phytopharmacological potential as compared with their glucosylated derivatives.

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MESOPOROUS NANOSTRUCTURED CERIA-TITANIA MIXED OXIDE DOPED WITH COPPER AS CATALYSTS FOR SUSTAINABLE ENVIRONMENTAL PROTECTION: EFFECT OF PREPARATION PROCEDURE

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In the last decade methanol has been considered as suitable clean and efficient alternative fuel because it can be synthesized from biomass and other waste products by well-known technologies and easily converted to hydrogen or synthesis gas in case of needs. On the other hand, volatile organic compounds (VOCs) emitted from various industrial processes and transport activities are considered as an important class of air pollutants, and catalytic combustion is one of the most promising strategies for their elimination at relatively low temperatures. An important step in the control of these catalytic processes is the development of highly efficient catalysts with controlled properties. Transition metal oxides are considered as appropriate alternative of noble metals and recently the main efforts are focused on the improvement of their electronic, surface and texture properties by the preparation of nanosized multi-component materials with well developed porous structure. The knowledge of the specific effects within the multi-component nanostructured metal oxides is prerequisite for the optimization of their properties. The aim of current investigation is to follow the effect of copper modification procedure of mesoporous ceria-titania oxides on their catalytic behaviour in methanol decomposition to CO and hydrogen as a potential alternative fuel and ethyl acetate oxidation as a representative VOCs. For the purpose of investigation the mesoporous ceria and/or titania oxides were synthesized by template assisted hydrothermal technique using CTAB as structure directed agent. For the first time a "chemisorption-hydrolysis" (CH) technique was applied for the copper loading on the ceria and/or titania oxides and compared with the conventional incipient wetness impregnation (WI) procedure. High surface area mesoporous ceria-titania binary materials can be successfully synthesized using template assisted hydrothermal technique. It was found that small additives of copper to ceria and/or titania oxides promoted their catalytic activity in total oxidation of ethyl acetate and methanol decomposition to syngas, but this effect is strongly influenced by preparation procedure. The applied "chemisorption-hydrolysis" technique provided formation of more homogeneously and finely dispersed copper species which also possess higher catalytic activity as compared to the conventional incipient wetness impregnation technique.

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MOLECULAR DESIGN AND CHEMICAL SYNTHESIS OF PEPTIDE INHIBITORS OF ANGIOTENSIN I CONVERTING ENZYME (ACE) FOR PREVENTION AND THERAPY OF HYPERTENSION

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Cardiovascular diseases (CVD), including hypertension and myocardial infarction, belong to the most worldwide common problems. Although synthetic antihypertensive drugs are available on the market, nutritionists claim that peptides which lower blood pressure are safer than “traditional” drugs and can be used as preventive agents. Recently, there is some scientific evidence that diet has a direct relationship to cardiovascular diseases.

The most of peptides inhibitors of ACE are relatively short sequences containing from 2 to 12 amino acids. Among them di- and tripeptides are more favourable as potential functional food additives candidates due to their high antihypertensive activity and low bitterness. Fernandez et al. [1] emphasized the role of ACE inhibitor peptides as a target for drug design resulting from the function of ACE in cardiovascular and renal diseases.

The purpose of the present study was to investigate the short bioactive peptide as novel inhibitors of angiotensin-I converting enzyme, for which were predicted to possess better ACE inhibitory activity and lesser side effects.

For the synthesis of each of the target peptides: H-Val-Ala-Trp-OH, H-Val-Ala-Pro-OH, H-Leu-Ala-Pro-OH, H-Ile-Ala-Lys-OH, were used solid phase peptide synthesis (SPPS) and microwave assisted SPPS. The obtained reaction mixtures were purified by HPLC and characterized by UPLC-MS and NMR.

We determined the relative inhibitory activity of the synthesized peptides and compared it to the commercially available ACE inhibitor- Lisinopril®, using modified method of Jimsheena and Gowda [2].

It is assumed that the newly synthesized peptides will be good additives in functional foods of hypertension.

References:

1. Fernandez J.H., Neshich G., Camargo A. C.M. (2004) Using bradykinin potentiating peptide structures to develop new antihypertensive drugs, Genet Mol Res, vol. 3, pp. 554–63.
2. Jimsheena V.K., Gowda L.R. (2009) Colorimetric, high-throughput assay for screening Angiotensin I-converting enzyme inhibitors, Analytical Chemistry, vol. 81, pp. 9388-9394.

ANTIMICROBIAL POTENTIAL OF BIOLOGICALLY ACTIVE COMPOUNDS DERIVED FROM BULGARIAN TOAD SKIN SECRETION

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Over the past decade, amphibians (frogs and toads) particularly their skin secretions, have provoked an increasing interest because of their potential for drug development.

The goal of this study is to elucidate the antimicrobial potential of skin gland secretion isolated from Bulgarian *Bombina variegata* toad. It is known that the skin secretions of *Bombina* species contain peptides and small proteins with different and interesting biological properties [1]. A solution of lyophilized skin secretions was analyzed by reverse phase high performance liquid chromatography (RPHPLC) on C_{18} analytical column (Vydac 238 TP, 25x4.6 mm). The antimicrobial potential of all samples was determined by the microdilution method described by Andrews J. [2], using 96-well standard microtiter plates. The obtained results showed that isolated bioactive compounds possess activity against Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* (NBIMCC 1390), Gram-positive bacteria *Staphylococcus aureus* (CIMB209), and fungus *Candida albicans* (NBIMCC74).

The determination of detailed peptides structures isolated from Bulgarian *Bombina variegata* toad skin secretion is on progress.

References:

1. Lai R., Liu H., Lee W. H., Zhang Y. (2002) A novel proline rich bombesin-related peptide (PR-bombesin) from toad Bombina maxima, Peptides, vol. 23, pp 437-442.
2. Andrews JM. (2001) Determination of minimal inhibitory concentrations, J of Antimicrob Chemother, vol. 48, pp. 5-16.

PREPARATION AND CHARACTERIZATION OF CELL WALL MATERIAL FROM ROSE HIP FRUITS*

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In the present study, plant cell wall material from rose hip fruits was isolated as alcohol insoluble solids. Chemical composition of cell wall material and initial fruits (edible part) were investigated and compared. The amounts of present intracellular compounds, such as polyphenols (flavonoids), pigments, lipids, vitamin C, etc. were removed in a different extent during hot alcohol/acetone treatment. Contrary, proteins, polysaccharides, and some polyphenols (condensed tannins) were co-precipitated due to dehydration effect of alcohol, which was the main factor that restricted the extractability of ‘contaminants’. In addition, carbohydrates (mainly pectins and cellulose) were found out to be the main constituents of the ‘purified’ rose hip cell wall preparation.

From our study on the use of AIS as method for plant cell wall preparation, several conclusions may be drawn. The choice of the initial plant material treatment is strictly individual and it should depend on the major chemical constituents. There is no almost perfect method for the simultaneous and complete removal of all interfering substances without affecting the interaction between cell wall components. An improved method for cell wall preparation could be suggested, but at the same time this will result in generation of artifacts, time-consuming, toxic risk, etc. Therefore, we consider that the preparation of CWM is matter of compromise with aims of investigation. To the best of our knowledge there are no published reports on the method for isolation of RH cell wall material as AIS. Moreover, the current study is among the few presented more detailed information about the chemical composition of fruit AIS.

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ISOLATION AND ANALYSIS OF CELL WALL POLYSACCHARIDES FROM ROSE HIP FRUITS*

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In an attempt to elucidate polysaccharides, cell wall material from rose hip fruits was fractionated by successive extractions with cyclohexane-diamino-tetra-acetate (CDTA), Na_2CO_3, 1M KOH, 4M KOH, and 4M KOH + borate to obtain a chelating agent-soluble pectin fraction, a dilute Na_2CO_3-soluble pectin fraction, a 1M KOH-soluble hemicellulose fraction, a 4M KOH-soluble hemicellulose fraction and a cellulose-rich residue, respectively. The CDTA pectin and Na_2CO_3 fraction consisted mainly of GalA (37%) followed by Ara, Glc, and Gal. The 1M KOH and 4M KOH fraction contained less GalA and more neutral sugars than CDTA and Na_2CO_3. Eighty-eight percents of GalA present in the cell wall material was recovered in the above mentioned two pectin fractions. The CDTA pectin was characterized as highly methyl-esterified one (68.6%), contrary to Na_2CO_3 fraction (19.4%). The two hemicellulosic fractions consisted of different molecular weight populations. They also differed in their monosaccharide composition. It was found that Ara, Xyl, Gal and Glc were the main neutral sugar constituents of these fractions. In 4M KOH + borate fraction dominated mainly Ara and Man in high amount. The final residue (α-CR7) contained 38% of cellulose, as some acid polysaccharides were enmeshed with the cellulose. The CDTA pectin possessed antioxidant activity probably due to other phytochemical components such as polyphenols and proteins.

Knowledge of polysaccharide composition would facilitate opportunities for more complete and competent industrial application of the fruits. Additionally, that information would be useful for investigation of relationship between structure and biological activity. To the best of our knowledge there are no published reports on successively extracted polysaccharide fractions from rose hip fruits.

Acknowledgements: The first author acknowledges with gratitude the technical assistance of chemist Irina Z. Yanakieva. He also would like to thank Prof. Romualdo S. Fukushima, PhD from a Departamento de Nutrição e Produção Animal, Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Brazil for kindly provided reference and helpful comments. This work was funded by project № DFNP-214/16.05.2016 “Characterization and biological activity of pectic polysaccharide from the cells walls of rose hip fruits” of Program for career development of young scientists, Bulgarian Academy of Sciences.

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A NEW APPROACH FOR SYNTHESIS OF NANOSTRUCTURED MESOPOROUS Ce AND Zr OXIDE MATERIALS AND THEIR APPLICATION AS CATALYSTS

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CeO_2-ZrO_2 system is one of the most studied mixed metal oxides in the literature due to its important role in the operation of automotive catalysts. To enhance the redox properties and thermal stability of pure ceria, zirconia is often mixed as an additive to form solid solutions. In this study novel nanostructured ceria-zirconia materials were synthesized in the presence or absence of bulky cetyltrimethylammonium bromide (CTAB) surfactant using an original approach combining the utilization of urea as a precipitator followed by hydrothermal treatment step at two different temperatures (373 K or 413 K). Selected supports (Ce/Zr = 3:7 or 5:5) were modified with copper and/or cobalt via incipient wetness impregnation from the corresponding metal nitrates. The obtained materials were characterized by X-ray diffraction, nitrogen physisorption, UV-Vis spectroscopy, temperature-programmed reduction (TPR) with hydrogen and their potential application in catalysis was tested in ethyl acetate combustion as a model reaction for total oxidation of volatile organic compounds. The results show that the addition of ziconia to ceria leads to improved ability for oxygen absorption/release observed with the mixed ceria-zirconia samples during the conducted TPR measurements resulting in enhancement of their redox properties in comparison with pure ceria. The use of urea only as both precipitator and template gives optimal textural and structural characteristics of the obtained mixed oxides, especially for the samples with Zr content above 30 mol %, while the additional presence of bulky CTAB molecules during synthesis seems to limit Ce-Zr interactions and thus has a negative effect on their catalytic performance. The mixed oxide samples prepared using higher hydrothermal treatment temperature (413 K instead of 373 K) possess improved textural characteristics favor the higher catalytic activity found with the mixed ceria-zirconia samples synthesized by using higher hydrothermal treatment temperature. The influence of the type of modifying transition metal is better distinguished for the 3Ce7Zr support, especially within 550-600 K range, showing that the addition of copper only is sufficient for achieving the best catalytic results. At the same time, the support with higher ceria amount (5Ce5Zr) favors the catalytic behavior of the cobalt-containing modifications as well due to enhanced Co-Ce interaction.

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PHTHALOCYANINE COMPLEXES OF LARGE IONS (Lu^{3+} and Sn^{4+}) *VS.* H_2-PHTHALOCYANINE: SYNTHESIS, PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES

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Phthalocyanine complexes (MPcs) have been extensively studied during last years as photosensitizers for photodynamic therapy (PDT). Their absorption in the far red spectrum and high level of the generated singlet oxygen and other photocytotoxic species have been widely thought that they can be ideal photosensitizers for PDT. MPcs possess a much higher extinction coefficient of the Q band between 680 - 720 nm, which makes them efficiently excitable directly through living tissue [1]. A limited number of metal phthalocyanines such as Al, Zn and Si phthalocyanines are currently accepted for clinical usage for cancer PDT.

The development of new MPcs coordinated with different ions especially with a high atomic number for the needs of PDT is an active research field [2]. As it is known that the coordination with central diamagnetic ions promotes the intersystem crossing and facilitates the photocatalytic process. A possible approach to improve the photosensitizing capability includes the synthesis of phthalocyanines containing large atoms such as tin (Sn4+ and Lu3+) as central ions.

The study presents several synthesis strategies for coordination of the large atoms such as Sn(IV) and Lu(III) in the cavity of the phthalocyanine molecule and investigation of the effects of these ions on the photosensitizing and photophysical properties of the phthalocyanines compared to the properties of the same metal-free phthalocyanine. Antimicrobial activity of the studied phthalocyanine derivatives suggests high impact of the both complexes towards drug-resistant bacterial pathogens.

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References:

1. Mantareva V., Durmuş M., Aliosman M., Stoineva I., Angelov I (2016) Lutetium(III) acetate phthalocyanines for photodynamic therapy applications: Synthesis and photophysicochemical properties, vol. 14, Photodiagnosis and Photodynamic Therapy, pp. 98-103.
2. Zhang X., Shao X., Tian H., Sun X., Han K (2013) Synthesis, fluorescence, excited triplet state properties and singlet oxygen generation of para-(tert-butylphenoxy) substituted phthalocyanines containing group IV A central elements, vol. 99, Dyes and Pigments, pp. 480–488.

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VALIDATION OF A METHOD FOR DETERMINATION *Pb* AND *Cd* IN FOOD SUPPLEMENTS CONTAINING *CRATAEGUS* AND *TRIBULUS TERESTRIS*, BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY

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Abstract

Nutritional supplements containing well-known and long-used medicinal extracts from medicinal plants *Crataegus sp.* and *Tribulus terrestris L.* have a beneficial effect on cardiovascular function, sexual activity, muscle strength, and energy production. Their effect is due to biologically active flavonoids and oligomeric procyanidins from *Crataegus* oxiacantha and furostanol saponins and flavonoids contained in *Tribulus terrestris* [1].

The objective of this work is to develop and validate a method for quantification of lead and cadmium in food supplements for the purpose of its application in the quality control of medicinal products and nutritional supplements containing medicinal herbs *Crataegus sp.* and *Tribulus terrestrisL*[2]. The following atomic absorption method parameters were defined: range, linearity, LOD, LOQ, reproducibility and accuracy. Combined and expanded uncertainties were calculated. As an analytical method, flame atomic absorption analysis was chosen because of its availability, easy calibration, lack of significant chemical interference, and non-selective absorption. The method of preparation of the samples is wet mineralization. The choice of the determinants of lead and cadmium is based on the fact that their content in plant extracts is subject to strict control because of their high toxicity.

Acknowledgements: We express our gratitude to INOLAB Ltd. for kindly supplied packages of food supplement "Macorvis" tablets.

References:

1.Tassell M, Kingston R, Giliroy D, Lehane M, Furey A (2010) Hawthorn (Crataegus spp.) in the treatment of cardiovascular disease. Pharmacognosy Review 4 (7): 32–41.
2.European Pharmacopoeia, 8th ed.: Appendix: Atomic Spectrophotometry: Emission and Absorption: 2.2.22., 2.2.23., EDQM, (2014).

SYNTHESIS OF 1,2-DISUBSTITUTED PLANAR CHIRAL FERROCENE DERIVATIVES *VIA* SULFONAMIDE-DIRECTED *ORTHO*-LITHIATION

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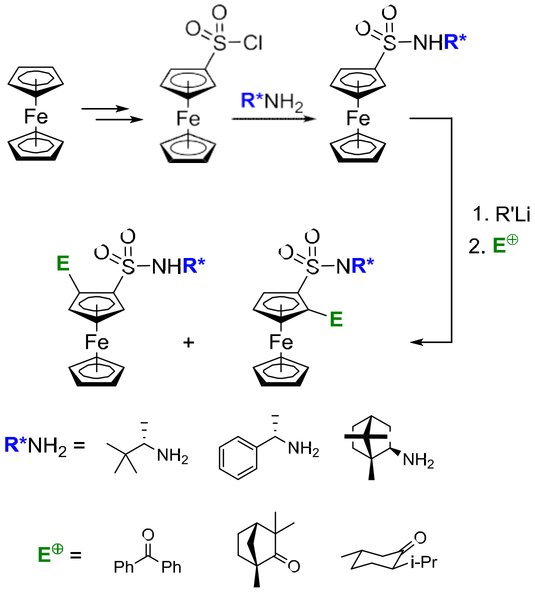
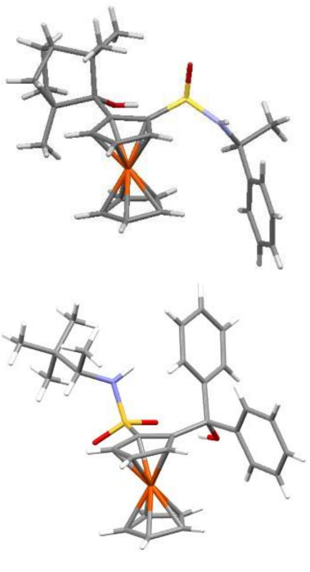
Planar chiral ferrocenes proved to be important ligands in catalytic asymmetric transformations in scientific research as well as in industry [1]. A plethora of different methodologies have been developed to access these molecules with a high degree of regio- and stereoselectivity [2]. Among the methods used to functionalize ferrocenes stereoselective *ortho*-metallation (e.g. lithiation) is undoubtedly the most frequently applied technique. Although a large variety of heteroatom-based chiral directing groups (e.g. amines, acetals, aminals, sulfoxides, oxazolines, hydrazones, imines, P-stereogenic groups) have been evaluated in the past, the potential of the sulfonamide group has not been exploited.

In this regard, herein is reported the synthesis and diastereoselective *ortho*-lithiation of chiral secondary ferrocenesulfonamides leading to new planar chiral 1,2-disubstituted derivatives. The catalytic activity of selected compounds was evaluated in the following model reactions: enantioselective 1,2- and 1,4-addition of diethylzinc to benzaldehyde and 2-cyclohexen-1-one, respectively; enantioselective reduction of 2-chloroacetophenone with borane-dimethyl sulfide.





Acknowledgements: The study was financially supported by the Program for career development of young scientists, BAS (DFNP-132); Bulgarian National Science Fund (Projects UNA-17/2005, DRNF-02/13/2009, DRNF-02/1/2009).

References:

1. Dai, L. X.; Hou, X. L. (2010). Chiral ferrocenes in asymmetric catalysis: synthesis and applications. John Wiley & Sons.
2. Schaarschmidt, D.; Lang, H. *Organometallics* **2013**, *32*, 5668 – 5704.

HEPATOPROTECTIVE AND ANTIOXIDANT PROPERTIES OF NOVEL HYDRAZONE DERIVATIVES OF THE N,N’-DISUBSTITUTED BENZIMIDAZOLE-2-THIONE SYNTHESIZED AS MELATONIN ANALOGUES AND STUDY OF THEIR MECHANISM OF ACTION

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In order to estimate the structure-activity relationship (SAR), DFT analysis was performed. On the base of the calculated reaction enthalpies of hydrogen atom abstraction (HAT) and single-electron transfer (SET) it could be concluded that the preferred mechanism of antioxidant action for the hydrazone derivatives of the N,N’-disubstituted benzimidazole-2-thione is SET.

Based on the structural resemblance of melatonin, series of novel hydrazone derivatives of the N,N’-disubstituted benzimidazole-2-thione have been synthesized as potential hepatoprotective and antioxidant drugs for the treatment of liver disorders. The compounds were tested on isolated rat hepatocytes and the antioxidant properties of the ones that exhibited lowest hepatotoxicity were evaluated by inhibition of the tert-butyl hydroperoxide (tert-BOOH)-dependent lipid peroxidation in induced oxidative stress. Three 3,3'-(2-thioxo-1H-benzo[d]imidazole-1,3(2H)-diyl)bis(N'-substituted-methoxybenzylidene) propanehydrazides and three 3,3'-(5-benzoyl-2-thioxo-1H-benzo[d]imidazole-1,3(2H)-diyl)bis(N'-substituted-methoxy benzylidene)propane-hydrazides showed significant cytoprotective and antioxidant effects similar to those of the referent quercetin. For further investigation of the antioxidant properties, the ability to inhibit the xanthine oxidase was evaluated in vitro.

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The poster was presented at First International Conference on Bio-antioxidants, 25.06.2017 - 29.06.2017, Sofia, Bulgaria

IN VITRO CYTOTOXICITY AND ANTI-HERPES SIMPLEX VIRUS ACTIVITY OF GRAPTOPETALUM PARAGUAYENSE E. WALTHER

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Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) are common human pathogens associated with orofacial infections, genital lesions and encephalitis. The toxic side effects and the emergence of virus strains that are resistant to the drugs, enhance the need for new effective compounds against viral infectious diseases.

Three main fractions were obtained - A (lipids), B (amino and organic acids, carbohydrates) and C (phenolic acids). The composition of each was determined by GC-MS analysis. The capacity for inhibition the lytic activity of HSV-1 Victoria strain and HSV-2 Bja strain and the reduction of viability of infected or uninfected cell cultures were defined by MTT assay. Data were used to calculate CC50. The cytopathic effect (CPE) was expressed as a percentage of the optical density of the sample compared with untreated virus-infected cells. Acyclovir was used as positive control.

Fractions C and A have not CPE on human cell lines RD and Lep and inhibited HSV replication in dose-dependent manner more efficiently against HSV-1, whereas their effect to HSV-2 were significantly lower. B fraction showed no antiviral effect.

The mechanism of the antiviral action of fractions C and A is not yet completely identified. Further studies are needed in order to verify which compounds could be responsible for this activity and how they exert their antiviral effects.

Постерът е представен на 3rd International Conference on Natural Products Utilization,
From Plants to Pharmacy Shelf, 18-21 October 2017, Bansko, Bulgaria

TAUTOMERISM OF INOSINE IN WATER: IS IT POSSIBLE?

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Inosine, 9-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3H-purin-6-one is a purine nucleoside that has hypoxanthine linked by the N9 nitrogen to the C1 carbon (β-N_9-glycosidic bond) of ribose. It is an intermediate in the degradation of purines and purine nucleosides to uric acid and in pathways of purine salvage. Inosine is commonly found in tRNAs and is essential for proper translation of the genetic code in wobble base pairs. The water-assisted proton transfer process in inosine was investigated using ab initio MP2 and SCS-MP2 quantum chemical approaches. It was found that in the gas phase and in water solution, the most stable tautomer for inosine is the 6-keto followed by the 6-enol form. Avalible X-ray data suggest that inosine can exist in two conformeric forms according to the ribose ring position – syn- and anti-conformation. The proton transfer for both confromeric forms of inosine keto and enol tautomers was considered. Potential energy surfaces of the "bare" finite solute-solvent clusters containing inosine molecule and five water molecules were explored, while within the second approach these clusters were embedded in "bulk" solvent treated as polarizable continuum (C-PCM/ SCS-MP2/6-31+G(d,p) level of theory). The rate constant is sufficiently large to generate the 6-enol tautomer. The analysis of the reaction profiles shows that the proton transfer processes occur through the asynchronous concerted mechanism.

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FORMAMIDE-BASED PREBIOTIC CHEMISTRY: ONE-POT SYNTHESIS OF NUCLEOBASES AND AMINO ACIDS

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In scenario framing the origin of life, the generation of precursors in abiotic conditions remains the major initial hurdle. Understanding the formation of biogenic molecules in abiotic conditions is a prerequisite in the origin-of-life studies. Determining the conditions allowing an efficient one-pot synthesis of the largest possible panel of biogenic compounds may shed light on the plausible scenario in which the processes that started life might have occurred. We report experiments describing the syntheses taking place from formamide. The formamide-based synthetic system proved to be particularly robust. Large panels of different compounds were observed – nucleobases (adenine, cytosine, uracil), amino acids (glycine, alanine), hypoxanthine, pterine, purine, urea and urocanic acid. Mechanism of reactions affording nucleic bases and amino acids were simulated by high-level ab initio quantum chemical methods.

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SYNTHESIS OF ENANTIOPURE STRUCTURALLY RIGID COMPOUNDS AS LIGANDS FOR ASYMMETRIC BORANE REDUCTION

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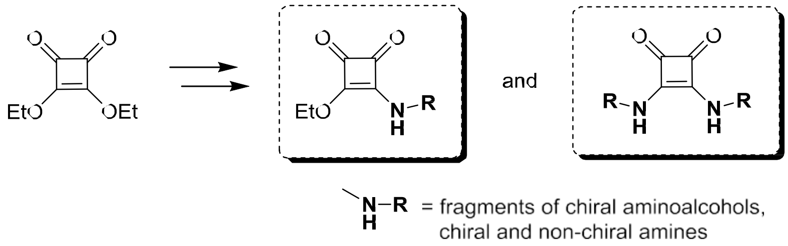
Synthesis of novel chiral compounds which can be used as catalysts for enantioselective reduction of ketones has been object of significant interest in recent years. Chirally modified boron complexes have been studied for their ability to induce enantioselectivity in reduction reactions. Asymmetric borane reduction is one of the most efficient ways for generating enantiomerically pure secondary alcohols. Remarkable success in this reaction has been achieved using chiral oxazaborolidines either formed *in situ* or prepared separately from natural compound-derived aminoalcohols such as amino acids, terpenoids etc. [1]. Interesting results have been reported recently in which squaric acid is used as a backbone to bind chiral aminoalcohol fragments. These structures can be used as chiral catalysts in asymmetric borane reduction [2].

We have prepared a series of compounds through combination of squaric acid and chiral amines or aminoalcohols, respectively. Within the current comparative study we are investigating the contribution of the squaric acid core (bearing different chiral aminoalcohol moieties) on the degree of enantioselectivity achieved by the reduction of ketones with $BH_3 \cdot SMe_2$.



All of the new compounds were isolated in very high yields and excellent purity. A series of 1D (^{1}H, ^{13}C, ^{11}B) and 2D NMR (^{1}H-^{15}N HMBC, ^{1}H DOSY) experiments were performed in order to investigate the reactivity and stability of the *in situ*-formed borane complexes.

The antitubercular activity of 5 of the synthesized compounds has been studied. The evaluation of their *in vitro* activity against *Mycobacterium tuberculosis* ($H_{37}Rv$) showed promising results in all cases.

Acknowledgements:

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References:

1. Cho, B. T. (2009) Chem. Soc. Rev. 38 443.
2. Wu, X. F., Min, C., Nyamzundui, E., Zhou, H. B., Dong, C. (2011) Tetrahedron Asymmetry 22 1640.

ULTRASONIC ACCELERATION OF ENZYME REACTION ON INSOLUBLE SUBSTRATES.

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Keratin is a structural and insoluble supercoiled protein which is mechanically resistant and recalcitrant to degradation by common proteolytic enzymes. Keratin has tightly packed molecular structure which is stabilized by cross-linking of disulphide bridges, hydrogen bonds or hydrophobic interactions [1]. Keratin is the major component of hair, wool, nails, hooves, claws, scales, horn, beaks and feathers factors. Feathers are made in 90% of keratin and constitute 5-7% of the total weight of an adult chicken. The global growth of the poultry industry generates over 4 million tons of feather waste annually [2].

Currently, different chemical methods for chicken feather hydrolysis, have found practical applications. However, as to extract soluble keratins and to achieve chemical cleavage of the disulphide bonds they are mostly employ strong acid and alkali. These chemicals used in chemical methods, such as sulfites, thiols, 1,4-dithiothreitol (DTT) or peroxides, are harmful, often toxic, and difficult to handle [3]. This generated the necessity of innovative solution to these problems.

Our laboratory scale studies found that enzyme performance can be significantly improved by introduction of ultrasonic energy during enzymatic feather hydrolysis.

References:

1. Brandelli, A. (2008). Bacterial keratinases: Useful enzymes for bioprocessing agro-industrial wastes and beyond. Food Bioprocess Technology. 1:105-116. DOI 10.1007/s11947-007-0025-y.
2.Suntornsuk W. (2003) Feather degradation by Bacillus sp. FK 46 in submerged cultivation, vol. 86. Bioresource Technology, pp. 239-234.
3. Wei, Z. (2012). Sustainable and practical utilization of feather keratin by an innovative physicochemical pretreatment: high density steam flash-explosion, vol (14). Green Chemistry, pp. 3352–3360.

POLYMERIC BRUSHES FOR TARGET pH-STIMULI DELIVERY OF PHOTOSENSITIZERS

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The drug delivery that is proper of releasing the active substance in response to stimuli has received much attention [1]. Since the pH gradients relevant for drug targeting can be found physiologically, the changes in pH are under research consideration. The gradients between normal tissues and some pathological sites, between the extracellular parts and some cellular compartments, and along the gastrointestinal tract are well studied. The normal blood has pH of 7.4, but extracellular pH values in cancerous tissues can be as low as 5.7. A greater pH differences can be found at the cellular level between the extracellular environment (pH 7.4) and intracellular compartments such as the endosomes and lysosomes (pH 4.5–6.5). This pH gradient is of particular importance due to several drugs and drug carriers are taken up by the endocytosis.

The formation, structure and drug release of new supramolecular assembles on the basis of polymeric brushes (with different chain length) conjugated with unsubstituted Zn(II) phthalocyanine were studied. The interactions on the basis of hydrophobic nature of PS were studied in dependence on pH values of the Buffer containing media by using fluorescence and transmittance spectroscopic techniques.

The hydrophobic Zn(II) phthalocyanine (Pc) was applied to two polymeric brushes published in Refs. [2, 3]. The results suggest that hydrophobic Pc forms conjugates with the both polymeric bushes. The studied pH-dependence showed in acidic media the Pc release and strong interaction below ~ pH 4 and after ~ pH 9. The binding sides between Pc and polymer PAT2/3 (2-1) can not be numbered for the concentration range typical for PDT applications.

Acknowledgements: The support by the National Science Fund (B02/9/2014).

References:

1. Pautov, V.D., T.N. Nekrasova, T.D. Anan’eva, T.K. Meleshko, D.M. Ilgach, A.V. Yakimansky, (2013), Polymer Science, Ser. A, 55, No. 9, pp. 535–539.
2. Pautov, V.D., T. N. Nekrasova, T. D. Anan’eva, T. K. Meleshko, D. M. Ilgach, A. V. Yakimansky, Intramolecular Mobility of Side Chains of Poly(methacrylic acid) in Regularly Grafted Copolyimides in Solution, Polymer Sci., Ser. A, 2013, 55(9), pp. 526–534.

Presented on the 17th Congress of ESP, Pisa, Italy, 4-8. Sept. 2017

PHTHALOCYANINE Zn(II) COMPLEXES WITH AMINOACIDS FOR PHOTODYNAMIC PROCESS

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The photodynamic process is based on effective action between a photosensitizer, atmospheric molecular oxygen and specific light from visible to near infrared spectra (630 – 850 nm). Thus results in generation of singlet oxygen and other reactive oxygen species which can oxidize the varieties of biomolecules with consequential cytotoxicity [1]. Phthalocyanines are promising photosensitizers for use in photodynamic therapy with an absorption wavelength within transparency windows of bio-tissues (630-850 nm), with favourable photophysical and photochemical properties of the high singlet oxygen yields [2]. Particular functionalization of phthalocyanine macrocycle with biologically-active compounds such as amino acids aims to improve the solubility as well as to enhance the cellular uptake and retention in target organelles selectivity. The study presents design, synthesis, photophysicochemical characterization and photodynamic efficacy of Zn(II) phthalocyanines (ZnPcs) with amino acids functionalization as photosensitizers for use in photodynamic applications. The tested tumor cells showed selective accumulation of the new conjugates compared to unsubstituted ZnPc which is an requirement for non-damages of the normal surrounding tissues.

Acknowledgements: Programme for career development of young scientists (No 150). The Project BG05M2OP001-2.009-0023, funded by the Operational Program "Science and Education Smart Growth", co-funded by the European Union through the European Structural and Investment Funds.

References:

1. Mantareva V, Angelov I, Worhle D, Borisova E, Kussovski V, (2013) Metallophthalocyanines for antimicrobial photodynamic therapy: an overview of our experience, 17, Journal of Porphyrins and Phthalocyanines, 1-18.
2. Wainwright M, Maisch T, Ninell S, Plaetzer K, Almeida A, Tegos G. P, Hamblin M, (2016), Photoantimicrobials—are we afraid of the light?, 17, The Lancet Inf. Diseases, 49-55.

Presented on the Congress of ESP, Pisa, Italy, Sept., 2017

ALGORITHM AND SOFTWARE FOR TESTING COMPLEX CHEMICAL KINETICS HYPOTHESES

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Chemical kinetics is the study of rates of chemical processes. However construction of mathematical models that adequately describes the rates of the chemical reactions set is not always easy task. While one can write simple kinetic equation derived from experimental data there is a possibility that such a reaction are not simple on molecular level. Most of the real chemical reactions are not large amount of identical elementary molecular acts but they rather are set of many different simple reactions interacting with each other by sharing common reactants and/or intermediates and redistribution of released and absorbed of that elementary reaction heat in the system. Unfortunately a set of simple chemical reactions suggested by our chemical intuition or by computational research, proposed to explain the conversion of reagents into products is not an easy task to be proven it in its details even though they explains overall experimental kinetics. In most cases it is also impossible to collect data for the concentration of every species in the system which leads to highly overdefined system when fitting the differential equation system of reaction set to the available experimental data. All of this leads to difficulties to compare the experimental kinetic data with data obtained by the computational chemistry.

In addition it should be mentioned that there is not ideal chemical reactor in real world because of wide variety of inhomogeneity that takes place in the real systems. Therefore different parts of reaction are effectively under deferent conditions that lead to unavoidable additional experimental errors, while obtaining data for concentration of the species.

Other complication in mathematical attempts to connect experimental data with suggested systems is that rate constants in such reaction sets often can differ in more than several orders of magnitude that leads to stiff system of differential equations in mathematical model that is difficult to be solved by ordinary numerical methods

A software packages that can be used to test such problems are available but they are expensive and because of their mostly mathematical orientation they are not very user friendly for chemists. In these packages the user must derive and set the differential equations system of the chemical reactions set by himself, but that is time consuming and is also a source of errors.

To facilitate process of testing of such computational or imaginary kinetic systems with real experimental data the automated algorithm and software described here is developed.

For this algorithm and software package a large set of heuristic algorithms is developed and implemented. A lot of heuristic algorithms are involved in the preprocessing steps in which chemical reaction set typed by user is transformed automatically to the set of deferential equations. The stiffness of the differential equations set is overcome by other heuristic algorithm which finds symbolically its partial derivatives, generating in this way pure analytical Jacobi matrix used in wide variety of the implemented integrators. Dealing with such large amount of symbolic equations led to developing of other heuristic algorithm that can solve symbolically every equation to ordinary number, further used in Integrators and optimisators in the software.

The software is tested in several real situations and also in imaginary situations with high computational complexity.

SLICE-SELECTIVE NMR APPROACHES FOR INVESTIGATION OF ASSOCIATION PHENOMENA

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Slice-selective NMR spectroscopy is one of the most commonly used techniques to detect spectra information from different parts of the sample and consequently – to investigate samples with different compositions along the NMR tube [1,2]. The inherently lower sensitivity, compared to conventional NMR experiments can be partially circumvented by the application of fast acquisition schemes, affording high quality NMR spectra in greatly reduced experimental times (**Fig. 1**). Here we present methodological and practical aspects of our studies in this field, including novel experimental approaches, investigation of supramolecular systems and distribution phenomena in water/chloroform samples.

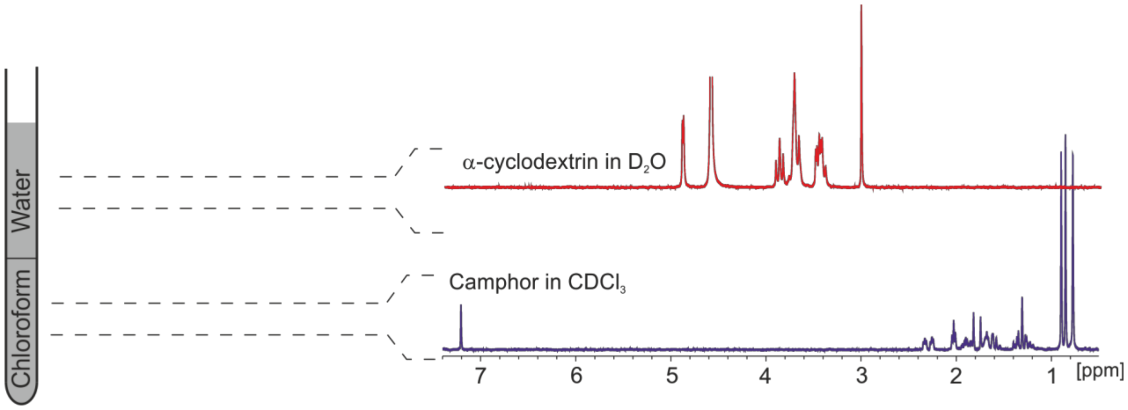

Figure 1. Slice-selective NMR spectra of water/chloroform sample

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References:

1. Y. Mitrev, S. Simova, D. Jeannerat (2016) „NMR analysis of weak molecular interactions using slice-selective experiments via study of concentration gradients in agar gels“, Chem. Commun., 52, pp 5418-5420.
2. Y. Mitrev (2017) “Slice selective NMR approach for investigation of distribution phenomena in biphasic samples”, Bul. Chem. Comm., 49, pp 65-69.

SYNTHESIS AND APPLICATION OF NEW CHIRAL AMINO-QUINOLINOLS

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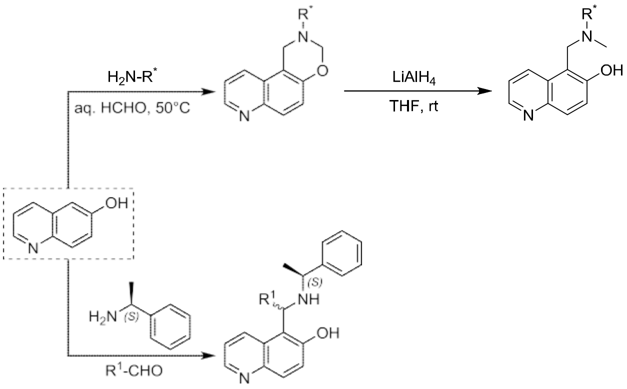
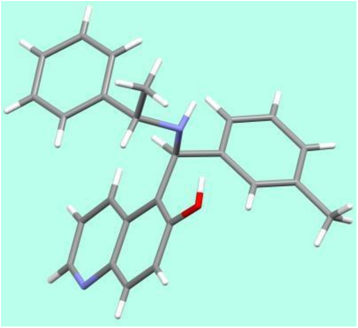
Multicomponent condensation reactions are a powerful method for the synthesis of organic compounds, since the products are formed in a single step and structural diversity can be achieved by variation of each component.

One of the important reactions in the field of multicomponent synthesis is the Betti reaction – condensation of 2-naphthol, aryl aldehydes, and ammonia. The modern development of the reaction includes the application of chiral amines replacing ammonia resulting in highly diastereoselective formation of chiral aminobenzylnaphthols. The latter type of compounds have been applied as efficient catalysts in the asymmetric synthesis.[1]

The variation of the components in the Betti reaction offers versatile opportunities for synthesis of novel structures for application in enantioselective processes, as has been recently demonstrated.[2]

We are presenting herein the synthesis of chiral 1,3-aminoquinolinols using different aldehydes, 6-hydroxyquinoline, as 2-naphthol analogue and chiral amines.





The stereoselectivity has been studied and the individual diastereoisomers have been isolated in pure form. The configurations of the newly formed stereogenic centres were determined through application of advanced NMR experiments and X-ray crystallography.

The aminoquinolinols obtained as pure diastereoisomers have been applied as catalysts in the enantioselective addition of Et_2Zn to aldehydes. In most cases excellent yields of isolated secondary alcohols and high degree of enantioselectivity were achieved (up to 99% ee).

Acknowledgements: Bulgarian National Science Fund (Projects UNA-17/2005, DRNF-02-13/2009; DRNF-02/1/2009).

References:

1. (a) Cimarelli, C., Palmieri G. *Chirality* (2009) *21*, 218; (b) Cardellicchio, C.; Capozzi, M.; Naso F. *Tetrahedron: Asymmetry* (2010) *21*, 507; (c) Szatmari, I.; Fülöp, F. *Tetrahedron* **2013**, *69*, 1255.
2. M. Marinova, K. Kostova, P. Tzvetkova, M. Tavlinova-Kirilova, A. Chimov, R. Nikolova, B. Shivachev, V. Dimitrov, *Tetrahedron: Asymmetry* **2013**, *24*, 1453–1466.

CHEMICAL PROFILING OF BULGARIAN ROSE ABSOLUTE (*ROSA DAMASCENA* MILL.) USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND TRIMETHYLSILYL DERIVATIVES

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Rosa damascena Mill. (Damask rose) is one of the most important plants belonging to the Rosaceae family, with a long historical use in the traditional medicine and as a valuable oil-bearing plant. Although, the genus Rosa consists over 200 species with 18 000 cultivars, only few of them have found industrial scale application for their fragrance and flavouring properties (*Rosa centifolia* L., *Rosa gallica* L., *Rosa alba* L. and *R. damascena* Mill.). Among them, *Rosa damascena* Mill., is considered to be superior in terms of the essential oil quality.

In addition to the traditional production of rose oil by steam-distillation, the rose scent could be extracted as a wax-like substance, called rose concrete by solvent extraction, which followed by ethyl alcohol re-extraction produces rose absolute. Following the production process, it is considered, that the rose absolute aroma reflects better the chemical composition of the rose flower

The geographic and botanical origin, environmental conditions, storage of the raw material and production method are some of the factors affecting the chemical composition and quality of essential oil and absolute and the sensorial and organoleptic characteristics of the products.

The aroma profile as well as the health influence are direct result of the chemical composition, this study could therefore provide a base for more adequate quality assessment, authenticity and allergen traceability in respect of the use of rose absolute in fine perfumery, natural cosmetics and clinical aromatherapy.

A comprehensive chemical profiling of Bulgarian rose absolute (*Rosa damascena* Mill.) was performed by means of GC/MS and GC-FID. A protocol for simultaneous analysis of compounds with high structural diversity and volatility, involving a multistep temperature gradient and trimethylsilyl derivatives was applied for this purpose. As a result 132 compounds, mainly mono- and sesquiterpenoids were identified. The main constituents, representing 80.0-95.5% of the total content of the detected compounds, were quantified by means of GC-FID. The developed approach was applied to rose absolute samples from Egypt and Morocco, as well. The specific chromatographic fingerprint, characteristic for the country of production (climatic-geographical origin) could be used for a fast classification of the rose absolute and for authenticity control. The accumulated information could have strong practical effect on the development of fast and high throughput methods for analysis.

ANTIOXIDANT ACTIVITY OF NATURALLY OCCURRING PHENOLS AND THEIR CORRESPONDING BIPHENOLS AS INDIVIDUAL COMPOUNDS AND IN MIXTURES WITH ANTICANCER DRUG 5-FLUOROURACIL

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The opportunity of combination therapies based on natural products and approved drugs, such as the combination of 5-fluorouracil (5-FU), an antineoplastic drug[1], with phenols has been successfully investigated (panel C). Although the growing interest in these mixtures and their potential use in medicinal applications, no information has been reported on their antioxidant activity in term of additivism or synergism that results from the combinations of these compounds. The present study aimed to investigate the antioxidant effect of 5-FU as individual compound and in equimolar mixture with some natural phenols and their corresponding biphenols. Electrochemical behavior of 5-FU and its antioxidant power on the oxidative environment produced by hydrogen peroxide (HP) have been assessed. Moreover, the eventual synergism produced by the co-presence of 5-FU and one of the most important physiological antioxidant compounds as ascorbic acid (AA) has been studied. Protective effect of some naturally occurring phenols and their biphenols as individual compounds and as equimolar binary mixture with 5-FU were studied during bulk lipid autoxidation (panel F). Synthesis of biphenols were carried out under sustainable conditions by C-C coupling reaction of the corresponding monomer (panel D and E).

The poster was presented at the First International Conference on Bio-antioxidants: “Natural Bio-antioxidants – as a base for new synthetic drugs and food additives/supplements”, 25-29 June 2017, Sofia, Bulgaria (Scientific Program, PP1).

ICBA 2017

ANTIOXIDANT ACTIVITY OF SELECTED O-METHOXYPHENOLS, BENZENEDIOL, BIPHENOLS AND TETRAHYDROXYBIPHENYL: THEORETICAL AND EXPERIMENTAL STUDIES

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A combination of theoretical and experimental approaches is applied to study and to explain the structure – antioxidant activity relationship for selected *ortho*-methoxyphenols (natural and natural-like phenols), phenol and benzenediol. The corresponding "dimers" (biphenols and tetrahydroxyphenyl) with selected positions of the phenol OH-group are handpicked in order to study the influence of the conformation and substituents in the aromatic ring on the antioxidant activity.

Chain-breaking antioxidant activities of compounds under study are determined from the kinetic curves of bulk lipid autoxidation. The molecular and biological properties (Lipinski Rule of Five) for all selected compounds are predicted by using Molinspiration program. Full geometry optimization of neutral molecules and their corresponding phenoxyl radicals for all compounds under study are obtained by using DFT (B3LYP/6-31+G**) calculations. Good correlation between experimental and predicted activity has been achieved which is helpful for the structure-activity relationship explanation.

The poster was presented at the Young Scientists School, First International Conference on Bio-antioxidants: "Natural Bio-antioxidants – as a base for new synthetic drugs and food additives/supplements", 25-29 June 2017, Sofia, Bulgaria (Scientific Program, YPP9).

The poster was awarded as one of the best poster presentations at the Young Scientists School on Bio-antioxidants.

ICBA 2017

SYNERGISTIC EFFECTS OF NEW ANTIOXIDANT COMPOSITIONS. ASSESSING THE ROLE OF EACH COMPONENT OF EQUIMOLAR TRIPLE MIXTURES

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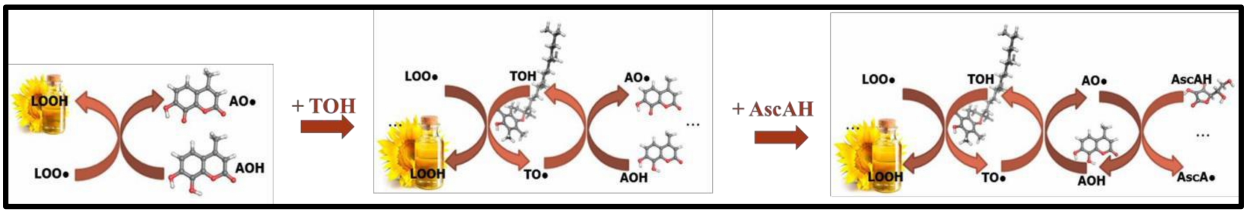
If two or more antioxidants are added to the oxidizing substrates, their combined inhibitory effect can be additive (summary), antagonistic (negative), or synergistic (positive). In the literature there is only one equation for synergism determination of binary mixtures. This equation has some disadvantages. First: in some cases it gives values over 100%. Second: when we would like to compare the experimental data from various experiments, made in different time and control samples, protection factor is more correctly to be used instead of induction periods. For that reason, we present for the first time new equations for assessing the synergism or antagonism of binary and triple mixtures. New antioxidant compositions - equimolar triple mixtures of selected hydroxyl coumarins with α-tocopherol (TOH) and ascorbic acid (AscAH) – have been prepared only for selected compounds with synergistic or at least additive effects in binary mixtures. The role of each component in the antioxidant compositions of triple mixtures has been identified based on new equations composed by us. All the triple mixtures demonstrated synergism as a result of a continuous regeneration of TOH from AOH and AscAH occurs.



The poster was presented at the First International Conference on Bio-antioxidants: "Natural Bio-antioxidants – as a base for new synthetic drugs and food additives/supplements", 25-29 June 2017, Sofia, Bulgaria (Scientific Program, PP17).

ICBA 2017

SYNTHESIS OF CAMPHANE-BASED COMPOUNDS AS POTENTIAL ANTICANCER AGENTS

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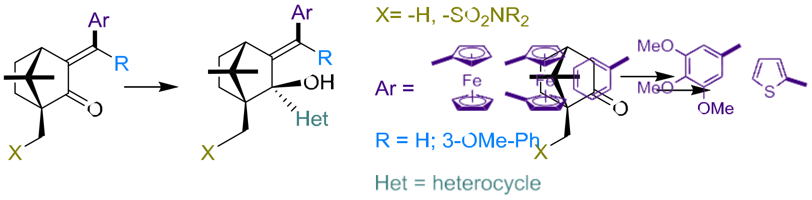
Camphor is a terpenoid found in the wood of the camphor laurel (*Cinnamomum camphora*) and other related trees of laurel family. It is a white crystalline substance and has been used for many centuries as a culinary spice, a component of incense, insect repellent and a flea-killing.[1] Camphor and its derivatives possessing bicyclic skeleton have shown promising biological activities as antioxidant, anti-inflammatory, anti-infective agents or anticancer.[2]

The treatment of cancer diseases remains a significant problem due to the observed multidrug resistance. Therefore, the preparation of new and more effective agents with potential anticancer activity is object of considerable synthetic efforts.

Herein we present a small library of (+)-camphor-derived compounds realizing structural diversity through variation of substituents (i.e. sulfonamide groups, ferrocenylmethylidene and arylmethylidene moieties and heterocyclic substituents). The obtained derivatives were tested against seven human cancer cells lines and two normal human cell lines in order to determine their activity against malignant cells. Some of them exhibit IC_{50} values below 10 μM in at least one of the cancer cell lines.[3]



Acknowledgements: Support of this work by the Bulgarian Scientific Fund (project MU0135/2008), by SCOPES program of Swiss National Science Foundation (project IB7320-111072/1) and by the Alexander von Humboldt Foundation is gratefully acknowledged.

References:

1. Hamidpour R, Hamidpour S, Hamidpour M, Shahlari M (2013) Camphor (*Cinnamomum camphora*), a traditional remedy with the history of treating several diseases. International Journal of Case Reports and Images 4: 86-89.
2. Shokova A, Kim JK, Kovalev VV (2016) Camphor and its derivatives. Unusual transformations and biological activity. Russian Journal of Organic Chemistry 52: 459-488.
3. (a) Kamenova-Nacheva M, Schröder M, Ugrinova I, Pasheva E, Slavchev I, Dimitrov V, Momekov G, Nikolova R, Shivachev B, Dobrikov GM (2017) Synthesis of ferrocenylmethylidene and arylidene substituted camphane based compounds as potential anticancer agents. New Journal of Chemistry: submit. (b) Kamenova-Nacheva M, Dobrikov GM, Dimitrov V (2016) Synthesis and catalytic application of ferrocene substituted camphane-based aminoalcohols and *S*-containing heterocyclic analogues. Tetrahedron Asymmetry 2**7**: 852-864.

Poster presentation at the 3rd International Conference on Natural Products Utilization - From Plants to Pharmacy Shelf, 18-21 October 2017, Bansko, Bulgaria

STEROIDAL AMINOBENZYLNAPHTHOLS AS PRIVILEGED LIGANDS FOR ASYMMETRIC REACTIONS

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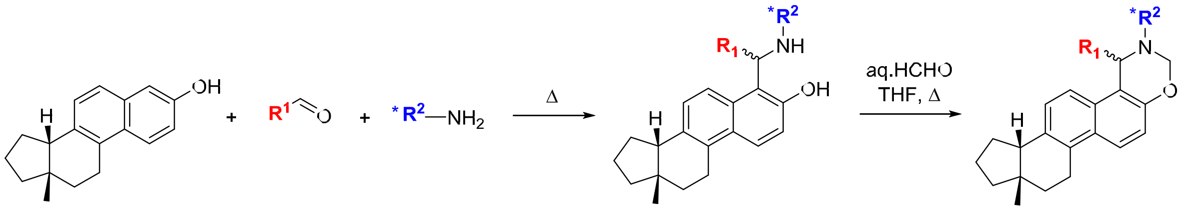
The process known as “Betti reaction” presents an easy to perform condensation between three components – 2-naphthol, aldehyde and amine. The condensation reaction may occur with remarkable diastereoselectivity by using of a suitable chiral amine and has significant synthetic potential for preparation of new complex chiral compounds through variation of the components [1]. These compounds are suitable building blocks for various asymmetric syntheses and could be applied as ligands in metal catalyzed processes.

We are presenting here the synthesis of new chiral aminobenzylnaphthol derivatives obtained through condensation between steroidal 2-naphthol analogue, functionalized aromatic aldehydes and chiral amines. The condensation reaction is stereoselective and the isolation of pure individual diastereoisomers has been achieved by easy to perform purification methods.



The data obtained from NOESY spectra allow configuration determination of new stereogenic centres by known configuration of the amine moiety. The 1,3-aminobenzylnaphthols form easily 1,3-dihydrooxazines which NOESY spectra additionally support the configuration determination. The configuration of newly formed stereogenic centres, determined by using of NMR-experimental data was confirmed by X-ray crystallography.

The newly synthesized chiral aminobenzylnaphthol derivatives have been applied as catalysts in the enantioselective addition of diethylzinc to aldehydes.

Acknowledgements: The study was financially supported by the Support Program of Young Scientists and Scholars of BAS (DFNP-149/2016); Bulgarian National Science Fund (DRNF-02/1/2009).

References:

1. Cardellicchio C., Capozzi M. A. M., Naso F., *Tetrahedron: Asymmetry* **21**, 507-517 (2010).

SYNTHESIS OF CHIRAL AMINOBENZYLNAPHTHOLS FOR CATALYTIC APPLICATIONS IN ASYMMETRIC SYNTHESIS

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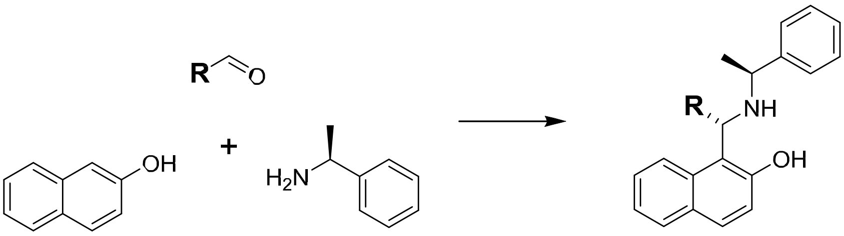
The three component condensation of Betti-type is an excellent tool for the synthesis of aminobenzylnaphthols by using readily available 2-naphthol, aldehydes and amines. It has been shown in recent years that this reaction can lead to structuraly diverse compounds, whereupon high degree of diastereoselectivity could be achieved throgh application of chiral amines [1]. The aminobenzyl naphthols synthesized have been recognized as bioligically active compounds and utilized as chiral ligands for asymmetric processes [2].

In this work we are describing the condensation of 2-naphthol, (S)-phenylethylamine and two space demanding aldehydes, fluorenecarbaldehyde and pyrenecarbaldehyde. The general interest was in realization of diastereoselective formation of aminobenzylnaphthols and isolation of pure diastereoisomers of these products.



Solvent free procedure was optimized to realize best yields. The configuration of new stereogenic centres was determined by using advanced NMR methods and was approved by X-ray crystallography. The new aminobenzylnaphthols were applied as catalysts for enantioselective addition of diethylzinc to aromatic aldehydes providing enantioselectivity of up to 93% ee.

Acknowledgements: Bulgarian National Science Fund (Projects UNA-17/2005).

References:

1. I. Szatmari, R. Sillanpää, F. Fülöp, *Tetrahedron: Asymmetry* **2008**, *19*, 612;
2. C. Cardellicchio, M. A. M. Capozzi, F. Naso, *Tetrahedron: Asymmetry* **2010**, *21*, 507

NEW EFFECTIVE ANTIOXIDANT COMPOSITIONS BASED ON BINARY AND TRIPLE MIXTURES

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Abstract

DL-α-tocopherol (**TOH**) and L(+)-ascorbic acid (**AscAH**) could be used as a strong base for further preparation of multi-component bio-antioxidant compositions with potential applications as food additives and in cosmetics.

Protective effects of binary and triple mixtures of **TOH** and ascorbylpalmitate (**AscPH,** the lipid soluble analogue of **AscAH**) with dehydrozingerone (a half of the curcumin molecule) during bulk phase lipid autoxidation were the focus of this study. Different effects in binary mixtures of **TOH** and **AscPH** were observed depending on the ratio (from 1:1 to 1:10) between the two components. It was observed that there was only additive effect (no synergism) for the ratios 1:5 (0.1 mM **TOH** and 0.5 mM **AscPH**) and 1:10 (0.1 mM **TOH** and 1.0 mM **AscPH**)**.** On the other hand significant synergism occurred between both components for the ratio 1:1 (38,1 %). For that reason equimolar ratios were chosen for studying not only the oxidation stability in presence of binary mixtures (1:1) of dehydrozingerone with α-tocopherol and ascorbylpalmitate but also their ternary mixture (1:1:1) in concentration 0.1 mM.

New reaction mechanisms are proposed, explaining the effects observed between individual compounds. The role of each component of the triple mixture is evaluated.

Acknowledgements: Authors thanks to the program for career development of young scientists BAS financing a project "*New effective antioxidant compositions on the base of binary and triple mixtures*" (126/26.05.2016).

COMPARATIVE STUDY OF ANTIOXIDANT POTENTIAL OF CURCUMIN AND ITS DEGRADATION PRODUCTS – VANILLIN, FERULIC ACID AND DEHYDROZINGERONE

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Abstract:

Curcumin is one of the most intensively studied biologically active antioxidants during the last decade. Its chemical instability and rapid degradation at physiological conditions (pH ≈ 7) was stated as the most important limitation for its potential applications. Numerous approaches have been undertaken to overcome the problem with the bioavailability of curcumin, including the use of: adjuvants such as piperine, liposomes, curcumin nanoparticles, phospholipid complexes and design of new structural analogs of curcumin [1]. On the other hand degradation of a compound does not necessarily lead to loss of its activity. Whether and how the degradation and oxidation pathways contribute to the biological and antioxidant activities of curcumin has also been discussed [2]. The aim of this study is to compare the antioxidant activity of curcumin with those of its degradation products (ferulic acid, vanillin and dehydrozingerone) identified by Wang *et al.* [3], when added in binary and ternary (triple) antioxidant mixtures. Lipid autoxidation has been used for assessing the chain-breaking antioxidant efficiency and reactivity of the phenols. The results obtained showed much stronger activity of curcumin than that of all the individual compounds and their binary and triple mixtures.

References:

1. Aggarwal, B.B., Sung, B. (2009) *Cell Press* **30**,85;
2. Schneider, C., O. N. Gordon, R. L. Edwards, P. B. Luis, (2015), *J. Agric.Food Chem.* **63**, 7606;
3. Wang, Y. J., Pan, A.-L. Chenq, L.-I. Lin, Y.-S.Ho, C.-Y. Hsieh, J.-K. Lin, (1997) *J.Pharm.Biomed. Anal.* **15**, 1867.

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ICBA 2017

CHAIN BREAKING ANTIOXIDANT ACTIVITY OF NEW SYNTHETIC ANALOGUES OF CURCUMIN AS INDIVIDUALS AND IN MIXTURES WITH CONVENTIONAL ANTIOXIDANTS

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Abstract:

The effectiveness of development of new biologically active compounds with antioxidant potential has been proved in the treatment of various diseases. The design of new synthetic analogues which combine minimal toxicity, higher stability and improved biological properties compared to the parent compound has always been of interest for medicinal chemistry. Curcumin is one of the most powerful natural bio-antioxidants but unfortunately its poor bioavailability and instability at physiological level is a serious disadvantage. Previously, we observed high antiproliferative and apoptotic activity of C_2-symmetrycal hydroxylated biphenyls curcumin-analogues against malignant melanoma [1]. We hypothesized that the presence of a biphenyl scaffold in a curcumin structure would control rigidity at the two aromatic rings and thus play an important role in enhancing antitumoral activity. Herein we have extended the synthesis of biphenyls-curcuminoids and their corresponding monomers. Chain-breaking antioxidant activity of the studied compounds is evaluated during bulk phase lipid autoxidation at 80 ^{o}C. Compounds that exhibit weak to moderate antioxidant activity were tested in mixture with stronger antioxidants. Synergism and antagonism of mixtures were assessed and discussed. All structures are optimized at UB3LYP/6-31+G(d,p) level in gas phase to explain the structure-activity relationships.

References:

1. Dettori, M. A., Fabbri, D., Pisano, M., Rozzo, C., Palmieri, G., Dess, A., Dallocchiop, R., Delogu, G. (2015), *Lett. Drug Des. Discov.*,*12*, 131.

The poster was presented at the First International Conference on Bio-antioxidants: "Natural Bio-antioxidants – as a base for new synthetic drugs and food additives/supplements", 25-29 June 2017, Sofia, Bulgaria (Scientific Program, PP2).

ICBA 2017

MECHANISM OF DIELS-ALDER REACTIONS OF CYCLOPENTADIENE WITH ALUMINUM CHLORIDE COMPLEXES OF ACTIVATED CYCLIC DIENOPHILES. A COMPUTATIONAL STUDY.

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

We study computationally the Diels-Alder reactions of cyclopentadiene and the complexes of aluminum chloride with four cyclic dienophiles in which the reactive double bond is incorporated in a cyclopentene or dihydrofuran ring and activated by an exocyclic carbonyl group. All studied reactions have moderate to high preference for the respective *exo* products [1]. Calculations are performed at the 6-311G(d,p) basis set using several DFT functionals from the Truhlar group. All possible conformers of the complexes, as well as solvent effects, are taken into account. Predicted stereoselectivities in solvent at the experimental temperatures are in very good agreement with experiment for the cyclopentene dienophiles studied. Predictions in cases of the dihydrofuran derivatives are somewhat overestimated in comparison to experiment, however correctly reproducing the observed selectivity change trend. Reaction mechanisms are explored by complete intrinsic reaction coordinate, IRC, analyses of the located transition structures, TSs. Most of the studied reactions have a concerted, although asynchronous mechanism. In a few cases the located TSs are even more asynchronous and calculations predict a two-step mechanism. Energies of the located respective intermediates and subsequent second TSs in the latter cases unequivocally indicate that the first step of the reactions is rate-determining.

Acknowledgements: A part of the reported computations has been carried out on the MADARA cluster, IOCCP, acquired under Project RNF01/0110 (2009-2011) of the National Science Fund of Bulgaria.

References:

1. K. S. Kim, Y. H. Joo (1999) *Exo*-Selectivity in the Diels-Alder Reaction of Cyclopentadiene with Activated Cyclic Dienophiles in Flexible Conformation, *Bull. Korean Chem. Soc*. **20**, pp. 389-391

THE ROLE OF DFT PARAMETRIZATION IN PREDICTING TRANSPORT PROPERTIES OF ACENES AND THIENOACENES

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The molecular design of organic semiconducting materials envisaged for applications as organic light-emitting diodes (OLEDs), organic solar cells (OSCs), organic thin-film transistors (OTFTs), etc. needs correct description of molecular charge carriers transport properties. The theory should properly represent the internal reorganization energies associated with electron- and hole- adoption, charge transfer rates as well as exciton binding energies. All these characteristics require reliable determination of the vertical and adiabatic ionization potentials and electron affinities as well as the transport and optical gaps. Density Functional Theory (DFT) is the widely used prominent first-principle method with reasonable computational cost which performance and accuracy, however, strongly depend on the parametrization in the exchange-correlation (XC) functionals [1].

In the present study various functionals with constant fractions of Hartree-Fock (HF) exchange as well as functionals with mixture of HF and DFT exchange either at long, short, or both ranges, are applied for assessing the internal reorganization energies for holes and electrons and exciton binding energies of acenes and heteroacenes with four to seven fused aromatic rings. Discussion is also addressed to the role of the size of the systems and nature of the heteroatoms in these ladder-type structures [2]. Calculations with all DFT functionals show that extended conjugation lowers reorganization energies for hole and electrons as well as exciton binding energies. However, the amount of HF exchange introduced into the hybrid functionals leads to very appreciable changes in the reorganization energies for a given molecular length.

Acknowledgements: The author is grateful for the opportunity to use computing resources of MADARA HPC cluster, financed by the National Research Fund of Bulgaria under Project RNF 01/0110.

References:

1. Lee, J.-C.; Chai, J.-D.; Lin, S.-T., Assessment of density functional methods for exciton binding energies and related optoelectronic properties. *RSC Adv.* **2015,** *5*, 101370-101376.
2. Canola, S.; Pecoraro, C.; Negri, F., Modeling p-type charge transport in thienoacene analogs of pentacene. *Theor. Chem. Acc.* **2016,** *135*, 33-41.

A part of the results has been reported in the talk “On the Role of the DFT Paramatriaztion in Predicting Optical and Transport Properties of Conjugated Organic Molecules” at 10-th Chemistry Conference, Plovdiv University “P. Hilendarski”, October, 2016.

COMPUTATIONAL DESIGN OF NOVEL CLASSES OF CHROMOPHORES, RELATED TO ESIPT

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

An important class of xenobiotic dyes, BODIPY®, may be structurally considered as taking its origins from ESIPT precursors. The many advantages of these dyes, as well as some of their shortcomings as compared to earlier “classical” dyes could be derived from precursor properties. This may happen to be important to the computational design of novel metal complex materials. [1] The range and extent of property changes upon complex formation by proton transfer ligands is the reason for our interest in the basics of their tautomerism, electron spectroscopy and computational design. [2] We understand the design as a cycle of graphical presentation(s) of possibly unknown molecule(s), followed by computational modeling of prototropic tautomers and proton transfer in both ground S_0 and excited S_1 (and higher) excited state(s). Several hypothetical classes of molecules have been computationally considered, a couple of compounds have been synthesized, and observed electronic (absorption and emission) spectra have been registered and once again remodeled by quantum chemical computation,[3] to conclude the design cycle.

Acknowledgements: A part of the reported computations has been carried out on the MADARA HPC cluster, IOCCP, acquired under Project RNF01/0110 (2009 - 2011) of the National Research Fund of Bulgaria.

References:

1. O. Hubin, A. D. Laurent, D. P. Verkauteren, D. Jacquemin (2014) Investigation of ESIPT in a panel of chromophores presenting N–H…N intramolecular hydrogen bonds, J. Chem. Theory Comput., 10, 4574.
2. A. D. Laurent, D. P. Verkauteren, D. Jacquemin, P. O. Hubin (2014) Investigation of ESIPT in a panel of chromophores presenting N–H···N intramolecular hydrogen bonds, Phys. Chem. Chem. Phys., 16, 25288.
3. S. M. Bakalova, F. Mendicuti, O. Castaño, J. Kaneti (2009) Design and Photophysical Properties of a New Molecule with a N-B-N Linked Chromophore, Chem. Phys. Letters, 478, 206;
4. S. M. Bakalova, J. Kaneti (2017) Theoretical insights regarding the electronic spectra and proton transfers in a sensor molecule, Bulg. Chem. Commun. 49, Spec. Iss. G, 71.

THEORETICAL-EXPERIMENTAL APPROACH FOR STRUCTURE ELUCIDATION OF NOVEL PALLADIUM N-HETEROCYCLIC CARBENE COMPLEXES

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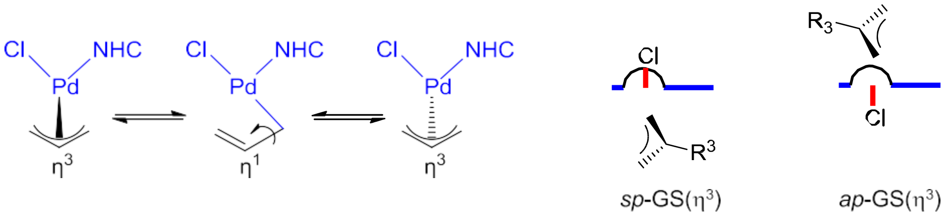
Theoretical-experimental approach was applied for structural elucidation of fluxional Pd(II) complexes bearing unsymmetrical N-heterocyclic carbenes (NHCs).The origin and energy barriers of series fluxional [Pd(NHC)(η^3-R-allyl)Cl] complexes derived from substituted imidazo[1,5-a]quinolin-1(2H)-ylidenes (see the figure)were examined and confirmed by combination of dynamic NMR and DFT studies[1].
Configuration of four platinum N-heterocyclic carbene complexes derived from substituted 1,8-naphthalimidewas proved by NMR spectroscopy. The DFT calculated thermodynamic parametersconfirm the observed stereochemistry of all complexes, which supports the statement that ligand exchange reactioncarries out under thermodynamic control [2].


Proved mechanism by DNMR and DFT studies

Acknowledgements: The financial support by the Program for career development of young scientists-BAS (DFNP-130/2016) is gratefully acknowledged

References:

1. Lyapchev, R. H., Petrov, P, Dangalov, M., Vassilev, N.G. (2017) Synthesis and structure elucidation of allyl Pd(II) complexes of NHC ligands derived from substituted imidazo[1,5-a]quinolin-1(2H)-ylidene, Journal of Organometallic Chemistry, 851, pp 194-209.

2. Dangalov, M., Vassilev, N.G., Petrov, P. (2017) Naphthalimide-based platinum (II) and palladium (II) N-heterocyclic carbene complexes: synthesis and structural elucidation, Bulgarian Chemical Communications, 49, B, pp. 42-49.

MICRO-RAMAN AND ATR-IR SPECTROSCOPY ANALYSIS OF PIGMENTS FROM THE MURAL PAINTINGS OF „THE ASSUMPTION OF ST IVAN RILSKI” CHURCH, RILA MONASTERY COMPLEX

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Rila Monastery, an eminent example of the architecture and fine arts of the Bulgarian national Revival, is a large Eastern orthodox monastery complex comprising in cultural, dwelling and farming buildings [1-3]. The complex includes several churches: the main monastery church "The Nativity of the Virgin", “The Presentation of the Virgin Mary" church, „The Assumption of St Ivan Rilski” church, “St. Peter and Paul” church in the nunnery “Orlica”, „The Assumption of The Virgin Mother” church in the nunnery “Pchelina”, “St. Evangelist Luka” church in the old hermitage etc. [2]. They were built over a long period of time – between the 15th and the 19th century and painted by famous Bulgarian artists, representatives of different schools [3].

Characterization of the pigment palette and binding materials in the mural paintings allow identifying the differences in the technology related to particular time periods and different artists. As a part of our ongoing study on the mural paintings in the Rila monastery, we conducted a micro-Raman and ATR-IR investigation on samples from „The Assumption of St Ivan Rilski” church painted in the first half of the 19th century. Micro-Raman and ATR-IR spectroscopy was used to provide molecular and structural information for the inorganic as well as for organic materials in a fast way without any sample preparation steps.
Ten samples were taken from different locations of the murals in order to include various colours from the background and the figures. The most significant pigments identified were: azurite, red ochre, green earths, red lead, lime white, carbon black.

Several samples of green, yellow and blue color gave strong fluorescence in the Raman study. In these cases, reliable identification was possible based on ATR-IR spectra. The obtained results provide useful basis for comparison with the mural paintings in main monastery church "The Nativity of the Virgin", painted by Dimitar Zograph and Ivan Obrazopisov in the period 1841- 1842, and by Zahari Zograph in 1844 year.

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The poster was presented at the 9th International Congress on the Application of Raman Spectroscopy in Art and Archaeology, Évora, Portugal, 24-27.10. 2017.

References:

1. Hristov H. (1959) The Rila Monastery (1st ed.) Publishing House of BAS, Sofia.
2. Koeva M. (2003) The Rila Monastery (2nd revised ed.) Borina Publishing House, Sofia.
3. Prashkov, L., Bakalova, E., Boyagjiev, S. (1990) Monasteries in Bulgaria (1st ed.) Spectrum Publishing House, Sofia, pp 218-242.

MULTI-ANALYTICAL STUDY OF THE PIGMENTS AND ORGANIC BINDERS FROM “ST. NIKOLAY” CHAPEL IN THE MAIN CHURCH "THE NATIVITY OF THE VIRGIN", RILA MONASTERY COMPLEX

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Multi-analytical approach based on several instrumental techniques is mandatory in the study of painting materials due to the complex nature of the samples [1]. Alterations in the composition resulting from natural aging and changes in the environmental conditions could further hamper the identification of pigments and binding media [2]. The use of an appropriate spectral database could considerably facilitate the identification, especially when spectra from real samples are provided along with the reference materials.

Recently we have developed a spectral database of art and archaeological materials focused on absorption FTIR spectra, total attenuated reflectance (ATR) spectra and Raman spectra of a number of pigments and dyes, adhesives, oils, resins, gums, bulk components, fillers, mixed materials, archaeological and art work samples [3].

Herein we report the identification of pigments and organic binders originating from the wall paintings in “St. Nikolay” Chapel, main church "The Nativity of the Virgin" of Rila monastery complex. The wall paintings were painted by Dimitar Zograph and his son Stanislav Dospevsky. Identification was achieved through ATR, micro-Raman and SEM-EDX measurements and spectral database application. Paint samples contained red lead, vermillion, red and yellow ochre, umbra and green earth pigments with egg binder. Among the green paint samples, emerald green mixed with egg was recognized, showing a good match with a previously studied sample from the altar. The blue paint contained smalt mixed with carbohydrate binder.

Acknowledgements: The study was financially supported by National Science Fund of Bulgaria (contract DFNI K02-15).

References:

1. FitzHugh E. W. (1997) Artists Pigments (1st ed.) Oxford University Press, Oxford, Volumes 1-4.
2. Van der Weerd J., van Loon A., Boon J. J. (2005) FTIR Studies of the Effects of Pigments on the Aging of Oil, Studies in Conservation, 50, 3-22.
3. Glavcheva Z. I., Yancheva D. Y., Kancheva Y. K., Velcheva E. A., Stamboliyska B. A. (2014) Development of FTIR spectra database of reference art and archaeological materials, Bulg. Chem. Commun., 46A, 164-169.

HPLC ANALYSIS OF POLYPHENOLS IN BULGARIAN WINE

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Wine is a widely consumed product for which strict safety standards and quality requirements related to geographical origin, varietal origin and production technology were introduced. In response, analytical methods were developed that can provide objective evidence of authenticity and a European databank was created with data for wines with declared geographical origin and varieties, which can to be used for checking and proving false claims.

In Bulgaria EU legislation for safety and quality control of food and beverages was adopted and analytical practice for safety control accepted in certified laboratories, but unfortunately virtually no accepted methods for verifying the authenticity of wines and proving geographical and varietal origin. Practically, no data were available for full characterization of the traditional Bulgarian wines and no reliable data for proving their geographical and varietal origin.

The objectives of our study are to provide a complete characterization of the traditional Bulgarian wines and offer a systematic approach to demonstrate geographical and varietal origin. In this direction we have developed a RP-HPLC protocol for analysis of polyphenols in selected Bulgarian wines using UV-detection. Totally 19 polyphenols have been targeted, namely: syringic acid, p-hydroxybenzoic acid, gallic acid, p-coumaric acid, caffeic acid, ferulic acid, resveratrol, (+)-catechin, (epi)catechin, kaempferol, quercetin, myricetin, cyanidin, delphinidin, malvidin, naringenin, hesperetin, 3.4-dihydroxybenzoic acid, rutin. In addition to that we have attempted to identify remaining polyphenols (as glucoside or aglycone) by using LC-MS.

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