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ХРОНИКА

Об итогах 23 Международной конференции по изопреноидам

(Минск, 4–7 сентября 2016 года)

В период с 4 по 7 сентября 2016 года в Минске на базе Института биоорганической химии НАН Беларуси состоялась 23-я Международная конференция по изопреноидам. В ней приняли участие более 100 ученых из Республики Беларусь и других стран.

Главная цель конференции – анализ современного состояния научно-исследовательских работ в области изопреноидов – широко распространенной и весьма многочисленной группы природных соединений, определение перспективных направлений их исследований и научно-технологического развития в интересах современных химико-фармацевтических, молекулярно-биологических, биомедицинских и агрохимических приложений.

В ходе конференции представлено 36 докладов, в том числе 8 пленарных и 11 ключевых, а также 52 стендовых сообщения. Пленарные и ключевые доклады были сделаны ведущими учеными-специалистами в данной области знаний из многих стран мира: Беларуси, Великобритании, Германии, Голландии, Италии, Польши, России, США, Украины, Чехии и Японии. Необходимо отметить активное участие в работе конференции молодых ученых, выступивших в дискуссиях и представивших интересные устные доклады и стендовые сообщения, лучшие из которых были отмечены наградами.

Работа конференции и проведенная общая дискуссия показали, что актуальными и перспективными направлениями исследований в области изопреноидов являются:

- поиск новых перспективных источников изопреноидного сырья среди объектов растительного мира и открытие новых структурных типов природных изопреноидов – основы для создания уникальных медицинских и сельскохозяйственных препаратов;
- открытие новых видов биологической активности, изучение особенностей физиологического действия, путей биосинтеза и биотрансформаций природных и синтетических изопреноидов, структурно-функциональных взаимосвязей, а также механизмов их участия в контроле процессов жизнедеятельности;
- разработка рациональных подходов к направленному химическому синтезу природных изопреноидов и их аналогов, микробиологический синтез и химическое моделирование ферментативного синтеза в интересах решения проблемы практической доступности физиологически значимых природных веществ;
- молекулярно-биологические, генно-инженерные, экологические и медицинские исследования изопреноидов.

Представленные на конференции доклады убедительно продемонстрировали высокий научно-методический уровень исследований, проводимых учеными разных стран, и достойное место науки Беларуси, находящейся в числе мировых лидеров. Работы выполняются в рамках национальных и межнациональных проектов и программ и вносят существенный вклад в понимание роли изопреноидов в функционировании живых организмов, в том числе в качестве регуляторов и участников обменных процессов.

В ходе всестороннего обсуждения докладов была подчеркнута целесообразность расширения и углубления исследований по вышеперечисленным перспективным направлениям исследований. Участники конференции отметили полезность и высокую научную значимость проведенного форума, способствующего установлению новых творческих связей для решения актуальных научных и практических проблем. Принято решение провести очередную 24-ю Международную конференцию по изопреноидам на базе Белостокского государственного университета (Польша) в 2018 году.

В настоящем номере журнала публикуются тезисы докладов 23-й Международной конференции по изопреноидам, не вошедшие в выпуск № 3 журнала «Весці Нацыянальнай акадэміі навук Беларусі. Серыя хімічных навук» за 2016 год, всецело посвященный материалам данной конференции.

🕮 LATE ABSTRACTS

🕮 CONJUGATES OF BETULINIC ACID WITH AMINES

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Betulinic acid is an important substance for designing of new drugs. Their derivatives have anticancer, antimicrobial, anti-inflammatory and anti-HIV activity. We synthesized derivatives of betulinic acid for subsequent tests of the biological activity.

We obtained three series of derivatives of betulinic acid. Two series contained conjugates of a hemiester with ethylene diamine, piperazine and spermine. The third series consists of conjugates with picolyl amines. (Fig. 1) The synthetic protocol for the first (I) and the second (II) series was based on three generally applied synthetic stages: (a) Coupling the hemiester with three Boc-protected amines: *N*-Boc diethyl amine, *N*-Boc piperazine and N^2,N^3,N^4-tris(*N*-Boc)-spermine by T3P. (b) Removing of benzyl protection group by catalytic hydrogenation with 1,4-cyclohexadiene as a source of hydrogen. (c) Removing the Boc protecting group by a solution of HCl in 1,4-dioxane. The third (III) series was the last one and for it, a simpler and more effective protocol was used, and its synthesis consists of only coupling of unprotected hemiester of betulinic acid with picolyl amines activated by T3P.

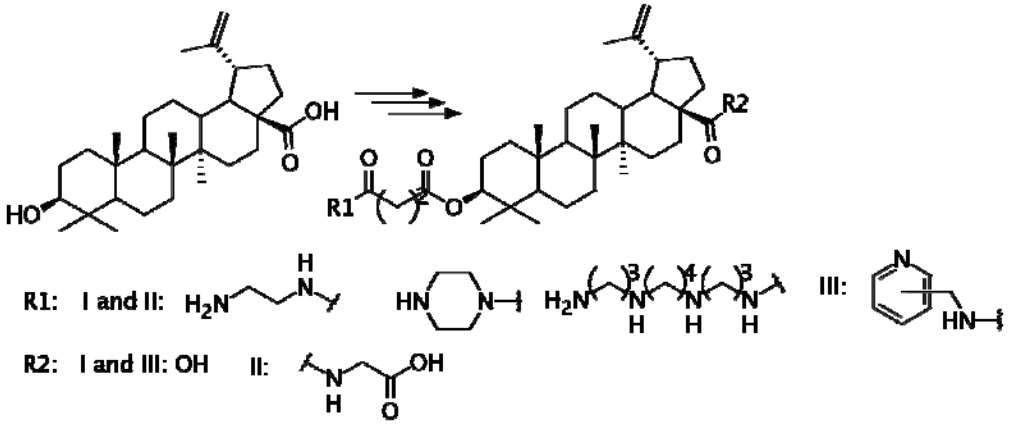

Fig 1.

Hemiesters were prepared by three different ways. The first hemiester was prepared from betulinic acid by a two step synthesis. It consists of protection of carboxyl functionality of betulinic acid by benzyl bromide and of a formation of hemisuccinate by a reaction with succinic anhydride in pyridine. The yields varied in the range of 80 and 95 %. The second hemiester was prepared by a six-steps process. Synthesis consist of a protection of 3-hydroxyl group by acetic anhydride, a formation of chloride by oxalyl chloride, attaching of ethyl protected glycine, removing of all protection group by lithium hydroxide, a protection of carboxyl group of glycine by benzyl bromide and a formation of hemisuccinate. The yield also varied in range of 80-95 %. The third hemiester was prepared by a reaction of betulinic acid with succinic anhydride. The yield was practically the same as in the previous ways.

All series of resulting derivatives were tested for cytotoxicity on the cells of human T-lymphoblastic leukemia, breast adenocarcinoma, cervical cancer, and also on normal human fibroblasts for comparison. Also they were tested against some microorganisms.

A financial support from the projects MSMT COST LD15012, a part of the COST Network CM1407 and TACR TA03010877 are gratefully acknowledged.

🕮 STEROID METABOLOMICS IN PREDICTION AND CLASSIFICATION OF PATHOLOGIES

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For decades, the steroid analysis serves in diagnostics of endocrinopathies related to deficiency of steroidogenic enzymes or to disturbed regulation of hypothalamo-pituitary-adrenal and hypothalamo-pituitary-gonadal axes. It is also useful in assessment of predisposition to steroid-related cancers and for their diagnosis. Modern techniques of instrumental analysis such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), gas chromatography (GC) or gas chromatography coupled to tandem mass spectrometry (GC/MS/MS) in combination with computer-aided data processing are well worked out and allow highly efficient detection and classification of various disorders particularly in neonatology and pediatrics. On the other hand, the altered steroid metabolome may be closely associated with additional pathologies. We have previously investigated the role of endogenous steroids and their diagnostic use in premenstrual syndrome[1] and, more recently, in further CNS diseases such as epilepsy[2], postpartum depressions[3], affective and anxiety disorders[4], autism[5], schizophrenia[6], multiple sclerosis[7] and Alzheimer's disease (AD)[8]. While in the case of postpartum depression the predictive value of circulating steroids was limited, in the remaining pathologies we found good or excellent predictivity, particularly in autism, AD and in affective and anxiety disorders. Besides the CNS disorders, we also the role of endogenous steroids in pregnancy complications such as preterm labor[9] and intrahepatic cholestasis of pregnancy (ICP)[10]. Our results obtained in the frame of ICP study indicated a potential use of steroid metabolomics in the diagnostics of ICP and gestational diabetes mellitus even when estimating a predisposition to these pathologies in out of pregnancy. Besides the diagnostic use, the steroid metabolomics contributed to explanation of pathophysiology of the above-mentioned disorders and allow to explain some aspects of human parturition and labor such as steroid feto-maternal differences in progestogens, estrogens and GABAergic steroids, which may be based on specific placental distribution of pluripotent 17β-hydroxysteroid dehydrogenases (HSD17Bs) and perhaps subfamily 1C aldoketoreductases (AKR1Cs)[11] (Fig. 1).

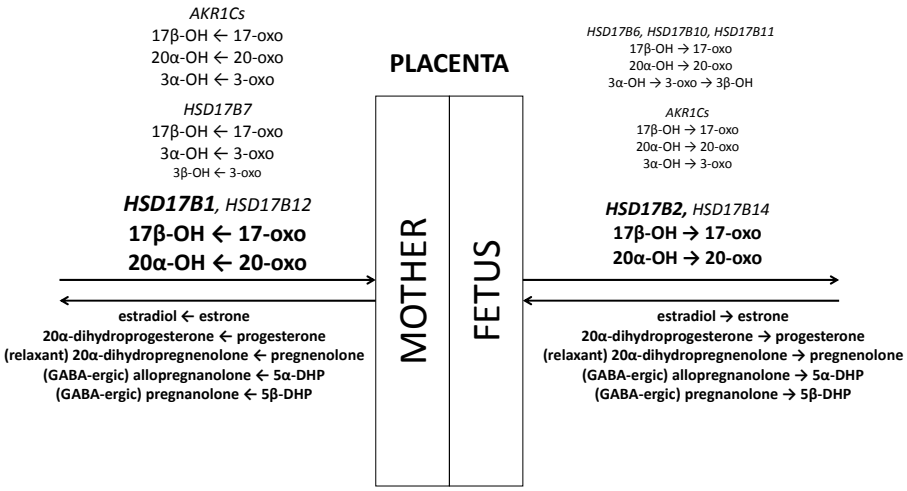

Figure 1. Simplified scheme of the effects of placental oxidoreductases in human late pregnancy

Besides, we found an association of ICP with persistently attenuated C17 hydroxylase-C17,20 lyase enzyme (CYP17A1) in the lyase step for so called steroid "backdoor" pathway, which shifts the balance between toxic sulfated pregnanediols (toxic for fetus but benign for mother) and nonthreatening sulfated C19 5α/β-reduced-17-oxo counterparts to the former noxious steroids[10] (Fig. 2).

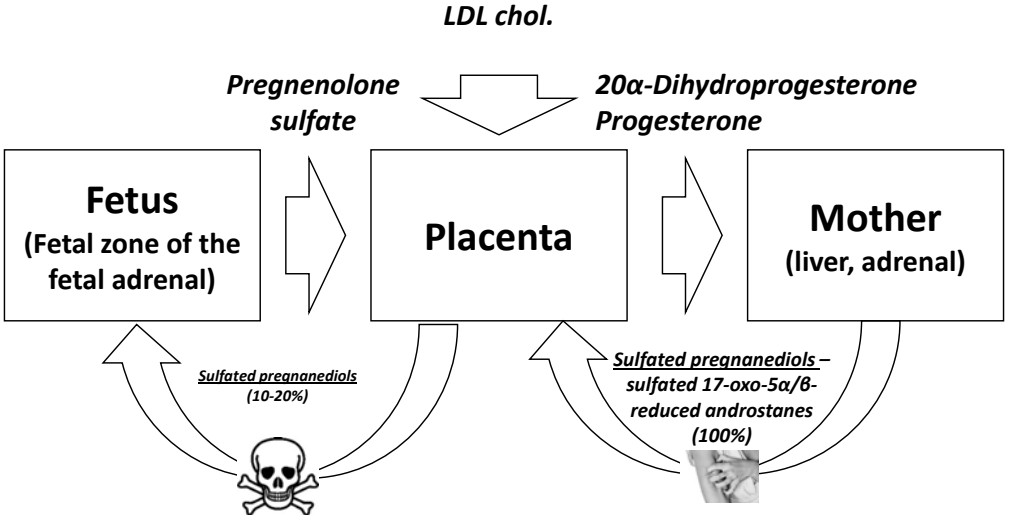

Figure 2. Simplified scheme of source, transport (mother→ fetus) and metabolism of pregnanediols in fetoplacental unit. Maternal *zona reticularis* (backdoor pathway); ICP patients: ↓CYP17A1 lyase activity → (↓reduced sulfated androstanes, ↑reduced sulfated pregnanediols - PD)

Our further results indicated lower activity of adrenal *zona reticularis* in female AD patients when compared with age- and gender-corresponding controls. Generally, our data indicate that the imbalance in activities of adrenocortical zones may participate in the pathophysiology of diseases, which are unrelated to steroid metabolism at first glance and our results exhibit that the steroid metabolomics may be valuable even in these cases.

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🕮 IMMUNOASSAY FOR DETERMINATION OF TRILOBOLIDE

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Trilobolide (Tb), a sesquiterpene lactone from Laser trilobum (L.) Borkh. (Apiaceae), is attracting much interest due to its extraordinary pharmacological properties. Mainly, its potency to irreversibly inhibit sarco/endoplasmatic reticulum Ca2+-ATPase has brought this natural product under the microscope of cancer treatment research. As long as there are still unanswered questions regarding its investigation, a need for novel analytical tools emerge. Since immunoassays often serve as powerful tools within investigation on natural products, we synthesized novel Tb-bearing conjugates and used them for immunization of rabbits and consequent development of immunoanalytical method.

Polyclonal antibodies raised against Tb-succinoyl-bovine serum albumin conjugate were determined as the most suitable for the development of chosen immunoassay format, indirect competitive enzyme-linked immunosorbent assay (ELISA), using avidin-biotin technology. The method was evaluated for its specificity, reproducibility, sensitivity and accuracy. Presented ELISA has demonstrated rather low cross-reactivity to other structurally related compounds not exceeding 6.1%. The system has also exhibited a satisfactory reproducibility expressed as intra- and inter-assay coefficients of variation reaching up to 9.7% and 11.4% respectively. Detection limit of 849 pg/mL and 50% intercept of 8.89 ng/mL suggest sensitivity of system.

The method was applied for Tb determination in three different plant parts of L. trilobum, while obtained results were in concordance with those obtained by conventional chromatography technique. Based on conducted experiments, we have proposed the use of presented ELISA for quantification of Tb in complex biological matrixes such as plant extracts.

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🕮 OXIDATION OF ISOPRENOID OLEFINS WITH BENZENESELENINIC ANHYDRIDE IN THE PRESENCE OF TMSOTF

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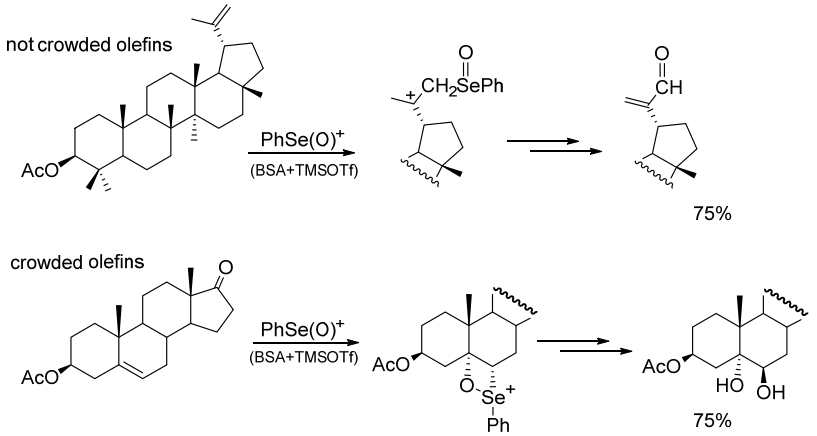
Polyoxygenated steroids is a family of compounds widely distributed in marine organisms.[1] Oxysterols attract much attention because of diverse biological effect in cell cultures.[2] The endogenous cholestane-3β,5α,6β-triol is reported as a cytotoxic.[3]

Here we present a new oxidizing reagent for olefins in the isoprenoid system.[4]

The reagent consists of benzeneseleninic anhydride and trimethylsilyl triflate. It is likely that the oxidation proceeds via a reactive species - benzeneseleninyl cation.



It has been shown that different products are formed with this generated *in situ* species depending on the specific structure of olefin. The 1,1-disubstituted olefins afforded mostly α,β-unsaturated carbonyl compounds. The sterically encumbered tri- or tetrasubstituted olefins yielded 1,2- or 1,4-dihydroxylated products, presumably via four-membered cyclic intermediates.

The dihydroxylated products are formed, presumably via the four-membered cyclic intermediate that is formed by a concerted [2+2] cycloaddition of a benzeneseleninyl cation to the olefin.

In the case of BSA/TMSOTf reactions with simple mono- or disubstituted olefins, the initial step is usually an electrophilic attack of selenium at the less substituted carbon atom. This step is followed by different consecutive reactions that cause the method to be rather ineffective.

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🕮 DESIGN OF NEW CHIRAL NOPINANE-ANNELATED PYRIDINES WITH 2 AND 4 PYRIDINE NUCLEI

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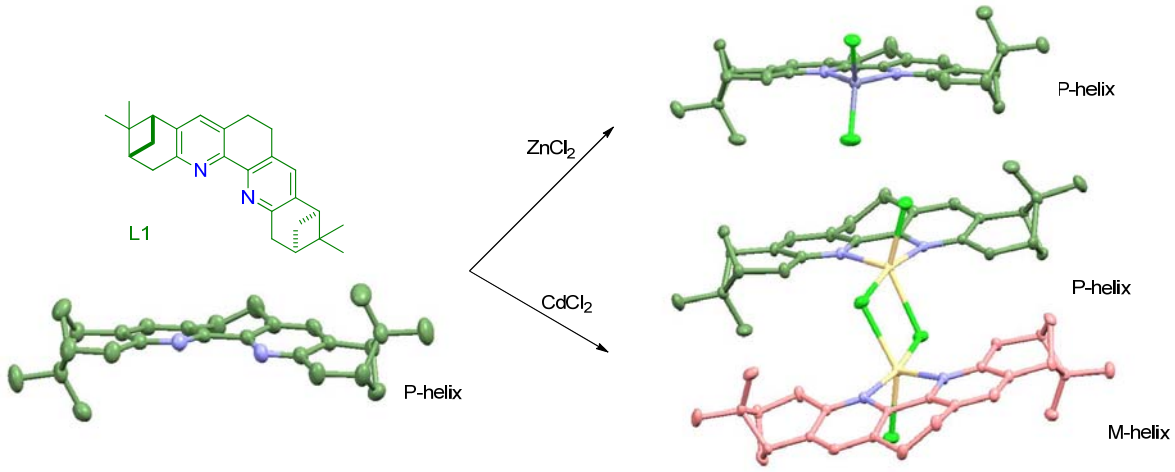
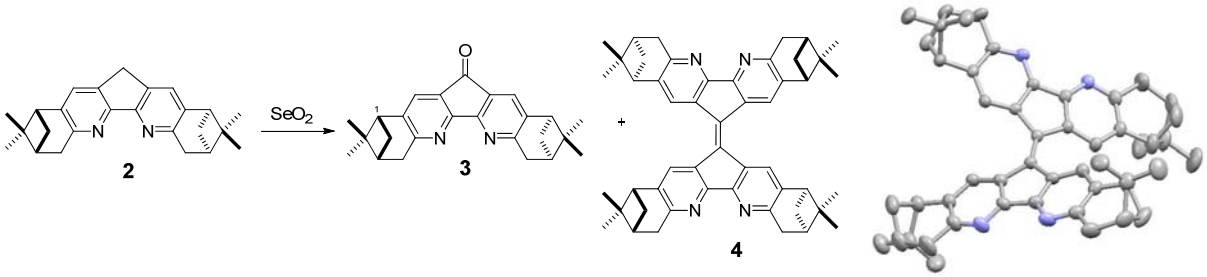
Fluorescent molecules of different chemical nature are successfully used in study of biological systems for a long time,[1] so design of new types of fluorescent chromophores, especially the chiral ones, are of special interest. We have described recently novel synthetic way to prospective ligand **L1**,[2] and its luminescent complexes with Zn(II) and Cd(II).[3] The bipyridine fragments of **L1** is quite flexible, so mononuclear complex **L1** with Zn(II) exists as *P*-helix in the solid state, whereas the crystalline dinuclear complex **L1** with Cd(II) exists as asymmetric dimer.



We have synthesized 5-membered analog **2** with a rigid bipyridine moiety and found it to be easily oxidized to give chiral diazafluorenone derivative **3** and tetrapyridine derivative **4** whose structures were proved by X-ray crystallography. Compound **4** has D_2-symmetry and in solid state exists as a single conformation.



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