🕮 ИЗОПРЕНОИДЫ: ТРАДИЦИЯ И СОВРЕМЕННОСТЬ

Н.Б. Хрипач, Ф.А. Лахвич, П. Драшар

[1]Институт биоорганической химии НАН Беларуси, 220141, Купревича 5/2, Минск; [2]University of Chemistry and Technology, Technická 5, 166 28 Praha 6 Dejvice
e-mail: nkhripach@iboch.bas-net.by, pavel.drasar@vscht.cz

Конференция по изопреноидам приближается к своему полувековому юбилею. История проведения форума берет начало на рубеже 60-х и 70-х годов прошлого столетия как национальная конференция по химии стероидов, организованная учеными Института органической химии академии наук Польши. Позднее по инициативе профессоров М. Коцура, В. Черни и В. Героута конференция стала совместным форумом чешских и польских ученых. Впоследствии тематика конференции была расширена путем включения исследований в области более широкого класса органических соединений – изопреноидов, среди которых стероиды занимают важное место. В 1973 году организатором очередной конференции выступил Институт органической химии и биохимии Чехословацкой академии наук, а местом проведения был выбран дворец Либлице в окрестностях Праги. С тех пор научные форумы проводились раз в два года поочередно в Польше и Чехословакии (впоследствии в Чехии) и традиционно собирали широкий круг специалистов различного профиля, связанных с исследованиями изопреноидов, из многих стран мира.

Столь длительная история и постоянный успех конференций были обусловлены рядом причин:

1) Предмет конференции – изопреноиды (терпеноиды) – широко распространенная многочисленная группа природных соединений, молекулы которых состоят из одного до 8 и более C_5-изопреновых блоков, соединенных между собой различным образом. Стероиды относятся к подклассу терпеноидов, содержат от 18 до 29 углеродных атомов и включают в качестве структурного элемента тетрациклический циклопентанопергидрофенантреновый (стероидный) скелет. Будучи природными веществами, содержащимися во всех живых организмах, изопреноиды играют важную роль в их функционировании, выступая регуляторами и участниками обменных процессов. К изопреноидам относятся многие витамины, а также стерины - один из ключевых элементов структуры клетки, стероидные гормоны человека и животных. Они ответственны за процессы репродукции, половой дифференциации, развития, адаптации, управляют минеральным и белковым обменом, нервной деятельностью, системой пищеварения, т.е. практически всеми жизненно значимыми функциями живого организма. Изопреноиды содержатся во всех высших растениях, и первые рецепты выделения содержащих терпеноиды эфирных масел из растений известны еще с дохристианских времен. Наиболее важными аспектами изопреноидов в практической области является их применение в качестве действующих веществ лекарственных препаратов, регуляторов роста и развития растений, использование в парфюмерии и пищевой промышленности в качестве душистых и пряных веществ. Для этих целей используются как выделенные из растений природные продукты, так и их синтетические аналоги, над созданием которых работают многочисленные коллективы исследователей-химиков.

С середины прошлого века начинает коренным образом меняться инструментальная база химического эксперимента в результате стремительного развития и внедрения аналитических методов, таких как хроматография, масс-спектрометрия, ЯМР-спектроскопия, оптическая спектроскопия и др. Становится возможным разделение сложных смесей, выделение в индивидуальном состоянии соединений, содержащихся в природных источниках в очень малых количествах, установление строения сложных органических соединений. Одновременно пришло осознание потребности общества в химических продуктах и технологиях. Начинался «золотой век» химии, который нуждался и способствовал развитию исследований во всех областях, и в особенности, в химии

природных соединений, обеспечивших формирование теоретического фундамента современной органической и биоорганической химии и увенчанных рядом Нобелевских премий.

Привлекательной стороной конференции является включение в ее тематику широкого круга вопросов, связанных с изопреноидами: от распространения в природных источниках соединений данного класса, полного химического синтеза, структурных модификаций и структурного анализа, до молекулярно-биологических, генно-инженерных, экологических и медицинских аспектов. Все темы обсуждаются в рамках общей дискуссии с участием представителей всех научных направлений, что позволяет рассматривать каждый предмет со многих точек зрения и дает возможность молодым исследователям сформировать широкое видение состояния и перспектив данной области науки. Возможно, что именно это обстоятельство регулярно собирает под знамена конференции большое число молодых ученых.

Начавшись на национальном уровне, конференция по изопреноидам очень быстро превратилась в общеевропейский форум, к которому присоединились ученые из многих стран мира. Среди участников конференций были лауреаты Нобелевской премии проф. Д. Бартон и проф. Р. Нойори, известные ученые:

Г. Адам (ФРГ), Дж. Апсаймон (Канада), А. Берч (Австралия), Бэкстрем (Швеция), Д. Виха (Польша), А. Даневски (Польша), В. Даневски (Польша), А. де Грут (Голландия), М. Гроен (Голландия), А. Дрейдинг (Швеция), Ф. Зилен (Голландия), А. Касал (Чехия), Е. Каспи (США), Дж. Конноли (Великобритания), П. Косински (Великобритания), С. Лей (Великобритания), Н. ДеЛука (США), К. Мори (Япония), К. Наканиши (Япония), К. Николау (США), Т. Норин (Швеция), Атта-Ур-Рахман (Пакистан), Сух Дев (Индия), Б. Трост (США), Г. Уриссон (Франция), Д. Хармата (Чехия), Д. Файкош (Чехия), В. Франк (Германия) и многие другие.

Постоянными активными участниками конференции были химики из союзных республик СССР: Белоруссии, Казахстана, Молдавии, России, Узбекистана, Украины и др. (впоследствии из соответствующих независимых государств), - лидеры и представители научных школ по химии природных соединений: Н.К. Абубакиров, С.Н. Ананченко, А.А. Ахрем, П.Ф. Влад, Г.Б. Еляков, А.В. Камерницкий, Ф.А. Лахвич, А.М. Моисеенков, И.В. Торгов, М.С. Юнусов и другие. Личное общение, научные доклады и дискуссии способствовали рождению новых идей, налаживанию творческого сотрудничества и дружеских отношений. Была подготовлена почва для создания интернациональных коллективов для выполнения масштабных проектов, финансируемых различными фондами Европейского Союза, другими международными организациями. Создалось трансграничное общество профессионалов, которое оформилось формально как «Изопреноидное общество», о чем было провозглашено в Праге на 22 конференции по изопреноидам, организованной в 2014 г.

Сегодняшний ренессанс в исследованиях изопреноидов связан с фундаментальными открытиями последней трети двадцатого века, которые многократно расширили представления о роли изопреноидов в живых организмах и функционировании экосистем, отразили их поистине неисчерпаемые возможности в решении самых актуальных проблем современности: борьбы с сердечно-сосудистыми, онкологическими и другими опасными заболеваниями, создания экологически дружественных агрохимических препаратов и технологий для сельского хозяйства, принципиально нового решения технических задач на основе моделирования процессов распознавания, рецепции и сигналинга, протекающих в живой клетке. Выбор г. Минска в качестве места проведения очередной 23 конференции является очередной вехой в развитии и интернационализации исследований изопреноидов.

🕮 ISOPRENOIDS: TRADITION AND MODERNITY

Natalia B. Khripach,[1] Fedor A. Lakhvich,[1] Pavel Drašar[2]

[1]Institute of Bioorganic Chemistry, NAS of Belarus, 220141, Kuprevich str., 5, Minsk, Belarus;
[2]University of Chemistry and Technology, Technická 5, 166 28 Praha 6 Dejvice
e-mail: nkhripach@iboch.bas-net.by; pavel.drasar@vscht.cz

Conference on Isoprenoids is approaching its half-century anniversary. The history of the forum starts at the turn of the 60s and 70s of the last century as national conference on steroids organized by Polish chemists from the Institute of Organic Chemistry in Warsaw. Later, the conference became a joint event of Czech and Polish scientists on the initiative of professors M. Kocór, V. Černý, and V. Herout. Subsequently, the conference topic was expanded by including research on the broader class of organic compounds - isoprenoids, among which subclass of steroids takes an important place. In 1973, the Conference was organized by the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences in the castle of Liblice in the neighborhood of Prague. Since then, the Conferences were organized biennial in Poland and Czechoslovakia (later the Czech Republic) alternately and traditionally brought together a large number of specialists in the field from many countries.

The long history of the successful meetings became possible due to several favorable conditions which were put together:

1) The subject of the Conference. Isoprenoids (terpenoids) is a widespread large group of natural compounds whose molecules consist of one to eight or more C5 isoprene units connected to each other in various ways. Steroids are terpenoid subclass which molecules are composed from 18 - 29 carbon atoms and include tetracyclic cyclopentaphenanthrene skeleton as a structural element. Isoprenoids are detected in all living organisms and play an important role in their functioning, being participants and regulators of metabolic processes. A number of vitamins, hormones, pheromones, allelopathins, receptor sensors as well as sterols, the key elements of cell structure, and many other physiologically active natural compounds belong to isoprenoids. They are responsible for the reproduction, sexual differentiation, development, adaptation, regulation of mineral and protein metabolism, nervous activity, digestive system, i.e. virtually all the vitally important functions of a living organism. Isoprenoids are found in all higher plants, and the recipes for isolation of enriched with terpenoids essential oils from plants are known since pre-Christian times. There are numerous fields of practical application of isoprenoids. They are used as active pharmaceutical ingredients, as plant growth regulators, in perfumery and food industry as a spice and perfume substances, etc. For this purpose both natural products and their synthetic analogues are used, and many groups of chemists are busy with making the latter ones.

2) Since the middle of the last century, the instrumental tools for chemical research were changed radically as a result of the rapid development and implementation of analytical techniques, such as chromatography, mass spectrometry, NMR spectroscopy, optical spectroscopy, and others. It becomes easier to separate complex mixtures, to isolate minor individual compounds from natural sources, to determine the structure of complex organic compounds. At the same time, the society realized the necessity in extension of chemical products use and technologies development. The "golden age" of chemistry started, which required and promoted the research in all fields of chemistry. The progress in studies of natural products has led to the creation of the theoretical fundament of modern organic and bioorganic chemistry and the results were awarded to several Nobel Prizes.

3) An attractive feature of the Conference is a variety of isoprenoid-related topics: from natural sources, chemical synthesis and structural analysis to molecular biological, genetic engineering, ecological, and medicinal aspects. All topics are considered by the specialists from different fields during common discussions offering a broad vision of a subject that is especially important for young

scientists for imaging the current state and perspectives of natural products chemistry. Probably, this is one of the reasons of substantial representation of young scientists among the participants.

Started at the national level, the Conference on Isoprenoids quickly turned into a pan-European forum, joined by scientists from many countries of the world. Among the conference lecturers there were Nobel Prize Winners, Prof. D. H. R. Barton and Prof. R. Noyori, and other prominent chemists, to mention only a few:

G. Adam (Germany), J. Apsimon (Canada), A. Berch (Australia), P. Baekström (Sweden), E. Caspi (USA), J. Connolly (Great Britain), A. Daniewski (Poland), W. Daniewski (Poland), A. Dreiding (Switzerland), J. Fajkoš (Czech Republic), W. Francke (Germany), A. de Groot (Netherlands), M. Groen (Netherlands), J. Harmatha (Czech Republic), A. Kasal (Czech Republic), P. Kocienski (Great Britain), V. Ley (Great Britain), N. de Luca (USA), K. Mori (Japan), K.Nakanishi (USA), K. Nicolaou, (USA), T. Norin (Sweden), G. Ourisson (France), Atta-ur Rahman (Pakistan), Sukh Dev (India), B. Trost (USA), J. Wicha (Poland), F. Zeelen (Netherlands). Permanent active participants of the conference were the chemists from the Republics of the USSR: Belarus, Kazakhstan, Moldova, Russia, Uzbekistan, Ukraine, and others (later from the corresponding independent States) - leaders and representatives of the scientific schools in chemistry of natural compounds: N.K. Abubakirov, S.N. Ananchenko, A.A. Akhrem, P.F. Vlad, G.B. Elyakov, A.V. Kamernitsky, F.A. Lakhvich, A.M. Moiseenkov, I.V. Torgov, M.S. Yunusov, and others. Personal contacts, scientific reports and discussions contributed to the birth of new ideas, establishing productive cooperation and friendly relations. It also enabled (and enables), otherwise possibly not simple, direct contact between the experienced decision makers and the youngest generation of scientists within and between disciplines. The conference has built a basis for assembling the international teams for the implementation of large-scale projects financed by various EU funds and other international organizations. It creates a cross-border community of professionals - "Isoprenoid Society", that was announced in Prague in 2014 on 22nd Isoprenoid Conference.

Nowadays, one can see a renaissance of isoprenoid research. It came on the wave of fundamental findings made in the last third of the XX century, which brought new knowledge about isoprenoid role in living organisms and ecosystem functioning, pointed to the inexhaustible potential of isoprenoids in solving the urgent problems of modern time: fight against cancer, cardiovascular and other dangerous diseases, creation of ecologically friendly preparations and technologies for agriculture, new technical solutions based on the modeling of recognition, reception and signaling in the living cell. The choice of Minsk as the venue for the 23rd Conference is a new milestone in the development and internationalization of isoprenoids' research and practice.

🕮 PLENARY LECTURES

🕮 THE DEVELOPMENT OF NOVEL METHODS FOR DIRECT OXIDATIVE COUPLING

Andrey P. Antonchick*

Max-Planck-Institute für Molekulare Physiologie, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany; Technische Universität Dortmund, Otto-Hahn-Strasse 4a, 44221 Dortmund, Germany
e-mail: andrey.antonchick@mpi-dortmund.mpg.de

C-H Bonds are ubiquitous in organic compounds and are among the least reactive bonds. Direct and selective methods of functionalization of simple compounds are an important and longstanding goal in chemistry. The direct transformation of C-H bonds into new carbon-carbon or carbon-heteroatom bonds is a fundamental challenge in organic chemistry. Direct oxidative cross-coupling between two non-functionalized compounds is an environmentally benign and economically attractive synthetic strategy.

Hypervalent iodine(III) reagents found application in direct oxidative, metal-free cross-coupling of non-functionalized compounds under mild reaction conditions. Recently, we developed a novel organocatalytic method of intramolecular C-H bond amination for intramolecular and intermolecular amination of simple arenes.[1-3] In subsequent studies using hypervalent iodine reagents, we developed a novel method for the formation of C-C and C-O bonds using non-functionalized compounds.[4-8]. The development of those methods and studies on the reaction mechanism will be discussed.

Cyclopropanes represent versatile synthons in organic synthesis with unique reactivity. Strained carbocycles are present in many natural, biologically and medicinally important products. The synthesis of the cyclopropane moiety has evoked considerable interest, which resulted in the development of different synthetic strategies. These methods require the application of reactive pre-functionalized reagents. Therefore, the development of novel practical methods for the straightforward synthesis of cyclopropanes using non-functionalized materials remains a considerable challenge and their elaboration is highly desired.

We developed a copper-catalyzed cyclopropanation of maleimides with acetophenone derivatives with broad scope.[9] This reaction represents an unprecedented example of copper catalyzed stereoselective synthesis of annulated cyclopropanes. Mechanistic studies revealed a novel reactivity for copper catalyzed radical reactions. This method was applied in furan synthesis using catalytic annulation of acetophenone derivatives and alkyl acetylenedicarboxylate with a broad reaction scope.[10] The operationally simple method offers direct access to multisubstituted furan derivatives. Furthermore, we discovered an extraordinary (1+1+1) cyclotrimerization for the cyclopropane synthesis.[11] The cyclotrimerization was applied in a stereoselective synthesis of small saturated carbocycles from non-functionalized acetophenone derivatives. A broad scope of the cyclotrimerization was demonstrated and detailed studies on the reaction mechanism were performed.

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🕮 CONVERSION OF STEROIDOGENIC DRUGS BY ADRENAL STEROID HYDROXYLASES AND PRODUCTION OF NOVEL METABOLITES

Lina Schiffer, Simone Brixius-Anderko, Frank Hannemann, Anne-Rose Müller, Anna Hobler, Josef Zapp[1], Jens Neunzig, Mario Thevis[2], and Rita Bernhardt

Institute of Biochemistry, Saarland University, Campus B2.2, 66123 Saarbruecken, Germany; [1]Pharmaceutical Biology, Saarland University, Campus C2.2, 66123 Saarbruecken, Germany; [2]Institute of Biochemistry/Center for preventive Doping Research, German Sports University Cologne, Germany
e-mail: ritabern@mx.uni-saarland.de

It was investigated whether human steroid hydroxylases are able to accept other steroidal compounds as substrates besides their physiological ones. Two classes of compounds have been investigated in detail: anabolic steroids and the well-known drug spironolactone and its metabolite canrenone. We demonstrated that the human cytochromes P450 CYP11A1, CYP11B1 and CYP11B2 which are involved in the biosynthesis of steroid hormones by catalyzing the side-chain cleavage of cholesterol and the final steps in the biosynthesis of gluco- and mineralocorticoids, respectively, show an additional capability to metabolize the xenobiotic steroid oral-turinabol (OT), which is a common doping agent. In contrast, microsomal steroid hydroxylases did not convert OT. Metabolites of OT conversion by the CYP11B subfamily members were produced at mg-scale with a recombinant *Escherichia coli*-based whole cell system and could be identified by NMR to be 11β-OH-OT for both CYP11B isoforms, whereby CYP11B2 additionally formed 11β,18-diOH-OT and 11β-OH-OT-18-al, which rearranges to its tautomeric form 11β,18-expoxy-18-OH-OT. CYP11A1 produces 6 metabolites, which are proposed to include 2-OH-OT, 16-OH-OT and 2,16-diOH-OT based on LC-MS/MS analyses.[1]

Moreover, also spironolactone and canrenone can be converted by CYP11B1 and CYP11B2, while no conversion of the selective mineralocorticoid receptor antagonist eplerenone was observed.[2]

These findings suggest that steroidogenic P450s can contribute to drug metabolism and should be considered in drug design and toxicity studies.

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🕮 TERPENES FROM MICROORGANISMS

Jeroen S. Dickschat

University of Bonn, Kekulé-Institute for Organic Chemistry and Biochemistry, Gerhard-Domagk-Straße 1, 53121 Bonn, Germany
e-mail: dickschat@uni-bonn.de

Terpenes are well known from plants, but only recent research revealed that they are also widespread in microorganisms including bacteria and fungi. Genome sequencing has uncovered the genetic information for terpene cyclases in many microorganisms which now allows for gene cloning

and heterologous expression to investigate the products of unknown enzymes. Today the products of at least 50 bacterial terpene cyclases have been identified with main contributions from my laboratory.[1]

The cyclisations of linear terpene precursors such as geranyl (GPP), farnesyl (FPP) or geranylgeranyl diphosphate (GGPP) proceeds by diphosphate abstraction followed by a cyclisation cascade via cationic intermediates and final deprotonation or attack of water. The detailed mechanisms of terpene cyclases are difficult to address. Possible methods to investigate the enzyme mechanisms are:

(i) enzymatic conversion of isotopically labelled precursors,
(ii) enzyme crystallisations and site-directed mutagenesis,
(iii) quantum chemical calculations.

We have used all these approaches to investigate the mechanisms of various bacterial and fungal terpene cyclases.[2-4] The results of our work will be discussed in the lecture.

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🕮 TOTAL SYNTHESIS OF ISOPRENOIDS USING ASYMMETRIC CATALYSIS

Adriaan J. Minnaard

Stratingh Institute for Chemistry, University of Groningen, The Netherlands
e-mail: A.J.Minnaard@rug.nl

Homogeneous catalysis, including asymmetric catalysis, plays an important role in natural product synthesis. This holds in particular for isoprenoids (terpenes) and polyketides as for those compounds carbon-carbon and carbon-hydrogen bond formation is key to build up the desired compound skeleton and stereochemistry is overwhelmingly important.

In our group we focus on several challenging aspects in the development of homogeneous catalysis and apply the methods in the synthesis of natural products. The formation of quaternary stereocenters and the stereoselective formation of tertiary alcohols have our particular attention. Recently we developed a strategy for the copper-catalyzed enantioselective addition of Grignard reagents to prochiral ketones.[1,2] Although the method has limitations in both the scope of Grignard reagents and the scope of ketones, as such the method is very effective as it provides a direct way to chiral enantioenriched tertiary alcohols using these most readily available organometallics. The method was applied in the synthesis of γ-tocopherol, a main component of vitamin E.[3] The chiral tertiary ether in the tocopherols is a *piece de resistance* in most syntheses of these compounds.

An alternative approach to tertiary alcohols is the use of enantioselective epoxidation followed by stereospecific ring opening, illustrated in the synthesis of the aggregation pheromone of the Colorado potato beetle.[4]

The formation of quaternary stereocenters (that is, carbon surrounded by four carbons) is approached by the enantioselective palladium-catalyzed conjugate addition of arylboronic acids to β-substitituted enones.[5] This leads to useful building blocks and this is illustrated in the efficient synthesis of enokipodin A,[6] a sesquiterpene, and in the synthesis of mastigophorene,[7] a terpene dimer that in addition possesses axial chirality.

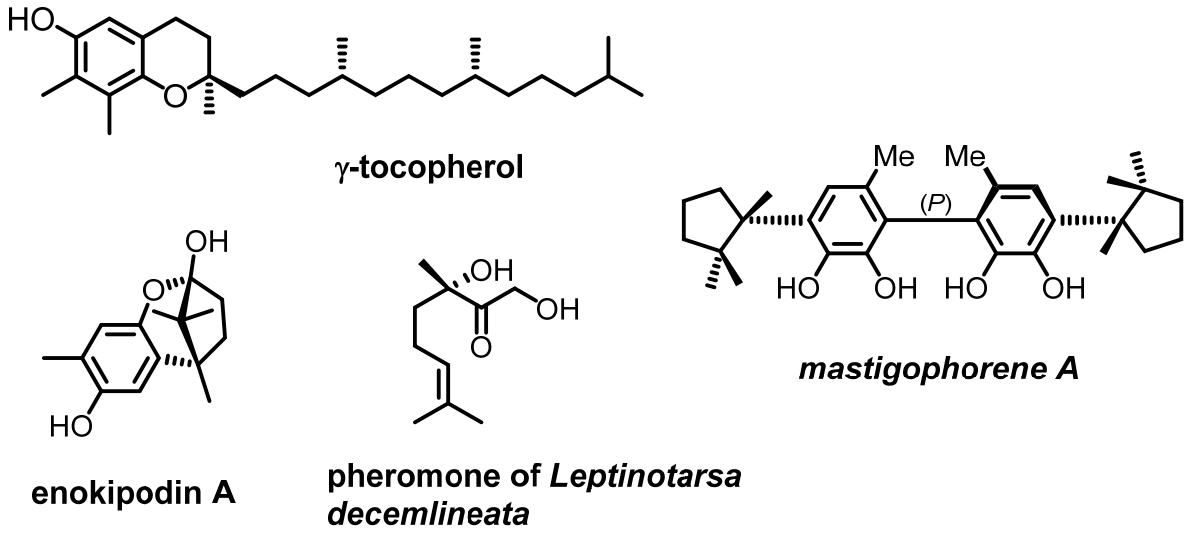

Figure 1.

An alternative approach to quaternary stereocenters is the asymmetric conjugate addition of organometallics to α-substituted enones.[8] The tetrasubstituted enolate resulting from the conjugate addition can be trapped with a carbon electrophile to provide the quaternary center. This method has been developed with some success and has been applied in the synthesis of the steroid skeleton,[9] based on approaches earlier reported in literature.[10] We showed in addition that the hydroxyl group in the A-ring could be introduced by iridium-catalyzed C-H activation.

Very recently we developed the first enantioselective synthesis of terpenes with the halimane skeleton, using yet another way to construct quaternary stereocenters, nl the Diels-Alder reaction. The compound tuberculosinyl adenosine was prepared in this way.[11] Tuberculosinyl adenosine was identified exclusively in pathogenic *Mycobacterium tuberculosis*, which makes it a *bona fide* candidate marker for the disease.[12]

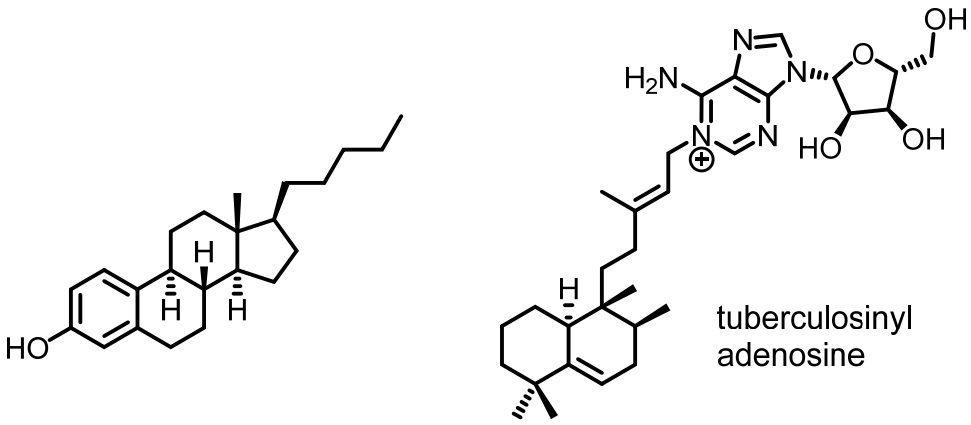

Figure 2.

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🕮 CHEMISTRY OF SEMIOCHEMICALS—A PERSONAL ACCOUNT—

Kenji Mori

The University of Tokyo, Tokyo, Japan
e-mail: kjk-mori@arion.ocn.ne.jp

Mori's works in the synthesis of bioactive natural products[1] will be reviewed with special emphasis on pheromone chemistry. The lecture will include the following topics.

(A) Structure determination by X-ray analysis (isoperiplanone-A[2]): Is it always correct (miyakosyne A[3]) ?

(B) Stereochemistry-bioactivity relationships among pheromones[4,5]: Are they always simple enough to regard only an enantiomer is bioactive ?

(C) A natural pheromone: Is it a stereoisomer with the most potent bioactivity (CH503[6,7]) ?

(D) A natural pheromone: Is it always a pure stereoisomer (tribolure[8]) ?

(E) How can we identify the correct structure of a pheromone (triglyceride pheromones[9,10] and a monoterpene pheromone of a mealybug[11]) ?

(F) Applications and perspectives of pheromone research.

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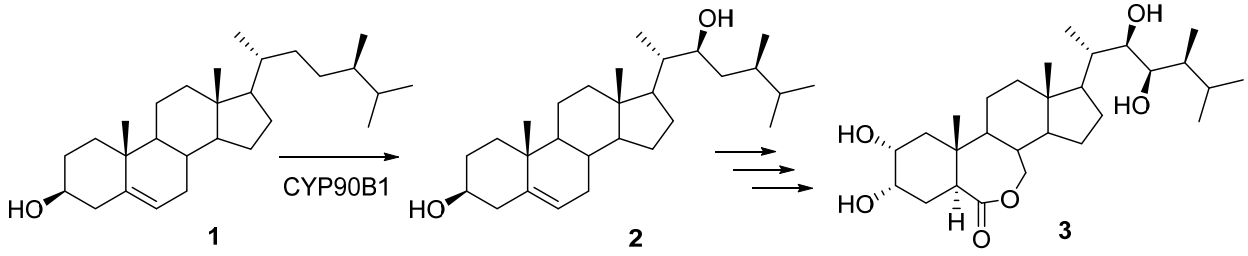
🕮 CRYSTAL STRUCTURE OF CYP90B1: IMPLICATIONS FOR BRASSINOSTEROID BIOSYNTHESIS

Keisuke Fujiyama[1], Masahiro Kanadani[1], Tomoya Hino[1], Masaharu Mizutani[2], and Shingo Nagano[1*]

[1] Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, Tottori, Japan, [2] Division of Applied Chemistry in Bioscience, Graduate School of Agricultural Science, Kobe University, Kobe, Japan
e-mail: snagano@bio.tottori-u.ac.jp

Brassinosteroids (BRs) are plant steroid hormones that promote plant growth and development.[1] The precursor of BR is campesterol (**1**), which is produced from squalene *via* cycloartenol. The most potent BR, brassinolide (**3**), contains many oxygen atoms, which are required for BR perception by it receptor and co-receptor kinases, BRI1 and BAK1, because many oxygen atoms in brassinolides are hydrogen bonded with BRI1 or BAK1. Most brassinolide oxygen atoms are introduced by cytochrome P450s (CYPs). CYP90B1 catalyzes the hydroxylation of C22 of campesterol (**1**), which is the first and rate limiting step of BR biosynthesis. Thus, CYP90B1 is the key enzyme for BR biosynthesis.[2] We have determined crystal structures of two inhibitor-bound forms of CYP90B1 (uniconazole (UCZ) and brassinazole (BRZ)). The overall structure of CYP90B1 has a P450 fold. The UCZ triazole coordinates to the heme iron and the chlorophenyl ring fits snugly into the hydrophobic pocket. We also determined the crystal structure of the BRZ-bound form at 2.3 Å resolution. Although the structures of UCZ and BRZ are similar, the conformations of these inhibitors bound to CYP90B1 show substantial differences. Moreover, there are significant conformational changes in CYP90B1 around the substrate binding pocket. A long loop before the A-helix moves further away from the active site and a C-terminal loop is relocated closer to the active site. Owing to these conformational changes, BRZ has a larger contact area with the enzyme than UCZ; therefore, BRZ has a higher affinity for CYP90B1 than UCZ. To understand the substrate binding mode and substrate selectivity, we performed a substrate docking simulation for CYP90B1. The side chain of campesterol runs parallel to the I-helix, which contains Thr315 that is essential for dioxygen activation by CYP. The C22 atom is 4.0 Å from the heme iron, which is a reasonable distance for the C22 hydroxylation by the enzyme. The steroid core of the substrate interacts with the hydrophobic wall of the large substrate binding pocket. The hydroxyl group, which is the only hydrophilic group on the substrate, is close to His385, which suggests a hydrogen bond between His385 and OH at C3 of the substrate. To verify the binding mode of cholesterol and campesterol suggested by the docking simulation, mutagenesis studies were performed on this enzyme. Replacement of most residues that were predicted to interact with the substrate resulted in the loss of hydroxylation activity, indicating that the substrate binding mode in CYP90B1 is similar to the docking simulation model and that the model can explain the regio- and stereospecific C22 hydroxylation by CYP90B1.



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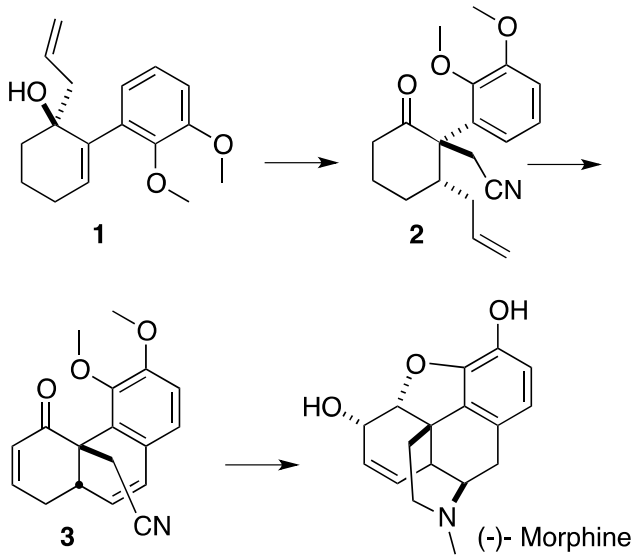
🕮 ORGANOCATALYSIS FOR THE 21ST CENTURY: SYNTHESIS OF MORPHINE

Douglass F. Taber, Sha Bai, and David A. Gerstenhaber

Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716 USA
e-mail: taberdf@udel.edu

The use of opium, the major alkaloid of which is morphine, dates back to at least 3,000 B.C. Crystalline morphine was first isolated by Friedrich Sertüner in 1806. The pentacyclic skeleton of morphine, including five stereogenic centers, an ether bridge and a piperidine ring, has offered a continuing challenge to the art and science of total synthesis. The first racemic synthesis was accomplished by Gates in 1952. The first practical racemic synthesis was by Rice in 1980, and the first enantioselective synthesis was achieved by Overman in 1993. In this work, we describe the practical multigram synthesis of the enone **3**, the tricarbocyclic core of (-)-morphine. The enabling step in the synthesis is the organocatalyzed allylation of 2-iodocyclohexenone by the Schaus protocol, that proceeds rapidly and in high enantiomeric excess at room temperature.



🕮 CHIRAL NANO- AND MESOSCOPIC STRUCTURES BY HIERARCHICAL SELF-ASSEMBLY OF STEROID-PORPHYRINS: 3D VS 2D AGGREGATION

Mariano Venanzi[1*], Donato Monti[1], and Pavel Drašar[2]

[1] Dept. of Chemical Sciences and Technologies, University of Rome 'Tor Vergata', Rome, Italy, [2] University of Chemistry and Technology, Prague, Czech Republic
e-mail: venanzi@uniroma2.it

Porphyrins are extremely versatile synthetic base materials. Fine tuning of their physicochemical properties can easily be achieved by functionalizing the core and/or the periphery of the electron-rich macrocyle, making derivatized porphyrins suitable for manifold applications as light-harvesting units in dye sensitized solar cells, chemical sensors, biomimetic catalysts, electro-and photo-active compounds in electronics and opto-electronics, sensitizers in photodynamic therapy of tumors.[1]

Intense research activity showed that, through hierarchical self-assembly, porphyrin aggregation can be driven to generate nano- and meso-structures of well-defined topology, such as nanoparticles, nanorods, molecular wires or ordered

monolayers. Aggregation can be controlled using porphyrin building blocks characterized by structural and chemical properties inducing a specific supramolecular organization, such as peripheral substituents featuring stereochemical information or soft/hard metal coordination to the core.[2]

A fundamental issue in the process of molecular self-assembly is the chirality generated during the building up of supramolecular structures. Chiral nano- and mesoscopic structures can derive from chiral groups functionalizing the porphyrin scaffold, transferring the stereogenic information of the single building block to the aggregated structure (sergeants-soldiers effect).[3]

In this contribution, the formation of chiral mesoscopic structures from porphyrin derivatives building blocks, characterized by functionalization of the *meso*-positions with multiple steroid groups will be described (Figure 1). Differences and similarities between aggregation in aqueous solutions (3D), essentially induced by hydrophobic effect, or by Langmuir-Blodgett techniques, in which the film formation (2D) is driven by increasing the surface pressure on the porphyrin film at the air/water interface will be discussed.

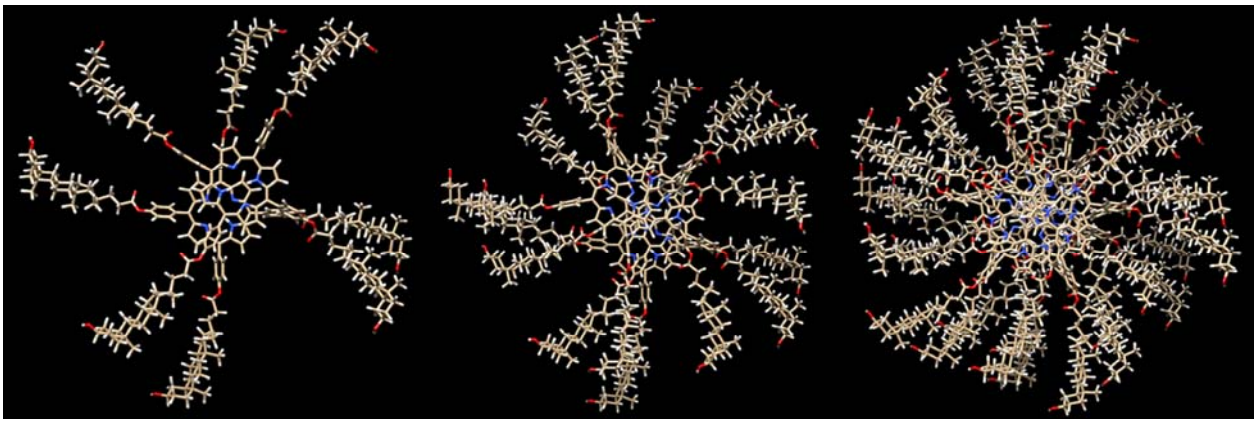


Figure 1. Stick representation of the minimum energy conformer of a tetrasteroidphenylporphyrin dimer, tetramer and octamer. C atoms: white; O atoms: red; N atoms: blue.

In particular, we will show how the number of steroid chains functionalizing the porphyrin scaffold can influence the aggregation process, leading to the formation of chiral mesoscopic structures. However, the morphology of the obtained supramolecular structures depend on the details of the intermolecular interactions established between the porphyrin units, involving electrostatic, van der Waals, and hydrogen bond interactions. Furthermore, solvation and hydrophobic effects strongly concur to stabilize the aggregated forms, determining the entropy balance of the process.

Kinetic experiments on the aggregation of steroid porphyrins also provide important insights on the mechanism of formation of nano- and mesoscopic structures. This process is strongly concentration dependent, and variation in the porphyrin concentration, even at micromolar levels, can deeply affect the aggregation mechanism. At low concentrations a typical two-step process occur, with an initial, faster formation of aspecific aggregates, followed by a slower growth of large aggregates. At relatively higher concentrations, very fast nucleation of small porphyrin clusters, is followed by a diffusionally-controlled growth of mesoscopic structures.

The Langmuir-Blodgett technique is most suitable to follow the formation of porphyrin films with increasing the surface pressure at the air water/interface. In particular, the surface pressure vs. molecular mean area isotherm allow to characterize the transition from the ‘gas phase’, i.e. non interacting porphryins, to the liquid condensed phase, i.e. collapse of the porphyrin film, through the liquid expanded phase, i.e. weakly interacting porphyrins (Figure 2).

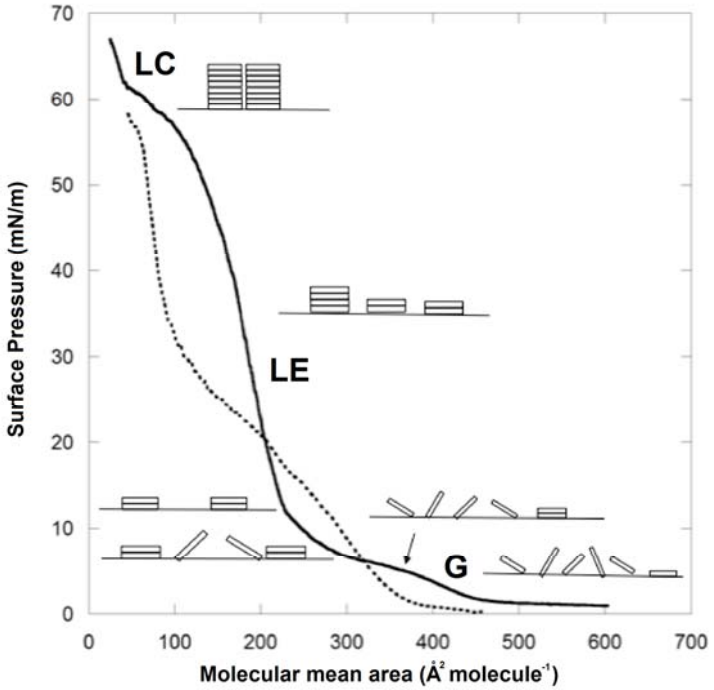


Figure 2. Surface Pressure vs. Molecular mean area isotherms of two tetrasteroid-porphyrins at the air/water interface. The two porphyrin compounds differ only by the number of hydroxyl groups in the steroid rings. G: gas phase; LE: liquid expanded phase; LC: liquid condensed phase. Sketches of the porphyrin organization in the different phases are also reported.

All these features will be amply discussed at the Conference, focusing on the molecular aspects of the aggregation process in aqueous solutions and at the air/water interface.

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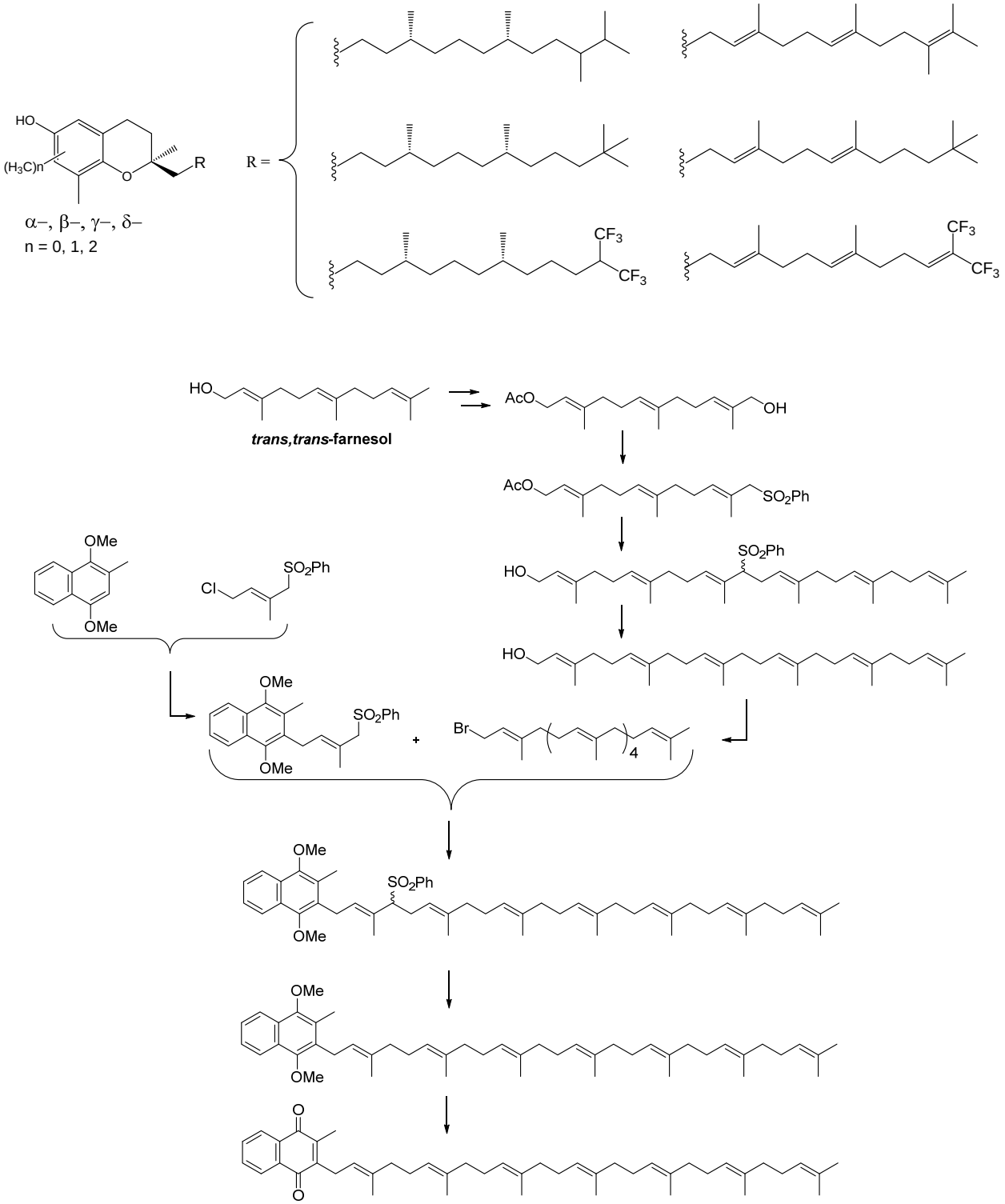
🕮 VITAMIN E AND K. ANALOGUES CONTAINING A MODIFIED LIPOPHILIC SIDE CHAIN

Stanisław Witkowski

Institute of Chemistry, University of Białystok, Białystok, Poland
e-mail: wit@uwb.edu.pl

Vitamin E includes eight chemically distinct isoprenoid compounds (tocochromanols) of plant origin. Tocopherols have fully saturated side chain attached to the 6-chromanol nucleus at the 2-position, while the side chain of tocotrienols contains three double bonds. Although all 8 vitamin E family members share a number of similarities, the fully methylated α-tocopherol (α-Toc) is the only one that is preferentially retained by the human body. It reveals the highest bioavailability due to its highest affinity to α-tocopherol transfer protein (α-TTP) and low rate of metabolism.[1,2]



Over past decades intense research efforts have been devoted mostly to α-tocopherol, while non-α-tocopherols have been regarded as redundant, and very little is known about their therapeutic value. Since recently a growing body of literature has been focused on other representatives of vitamin E, especially γ-tocopherol and tocotrienols, which often do not share biological activity of α-tocopherol.[3] γ-Tocopherol shows anti-inflammatory, anti-diabetic and anti-cancer activity, whereas, tocotrienols, especially γ- and δ-form, reveal numerous health-beneficial effects e.g. neu-

roprotective, cholesterol-lowering, anticancer etc.[4,5] Unfortunately, due to fast metabolic degradation of the hydrophobic side chain, the non-α-tocopherols are rapidly excreted from organism (as CEHCs), and its concentration in various tissues is very low compared to α-tocopherol.

In order to maintain a therapeutic level of tocochromanols the synthesis of analogs resistant against metabolic degradation was undertaken. Introduction of branching methyl groups or trifluoromethyl group should hinder or completely prevent cytochrome P-450-catalysed ω-hydroxylation and oxidation to 13'-carboxychromanol followed by five cycles of β-oxidation. Menaquinone-7 (MK-7) is a representative of vitamin K family, and necessary for blood coagulation, bone metabolism and cell growth.[6] The addition of MK-7 to diet substantially reduces the risk of bone fractures and cardiovascular disorders, which are both critical health issue worldwide and health care costs for their managements are high. The concentration of MK-7 in food products is low and there is a demand for supplementary MK-7. A Japanese fermented soybean (*natto*) is the richest source of MK-7. Nevertheless, the natural sources do not meet the demand of the market and the synthetic sources are desired. The presented stereoselective synthesis of MK-7 was achieved in a "1 + 6" convergent strategy by a condensation of the two building blocks, menadione monoprenyl derivative with hexaprenyl bromide using sulphone methodology.[7] The method enables production of high purity MK-7, which is suitable for supplementation and for therapeutic use.[8]

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🕮 KEYNOTE LECTURES

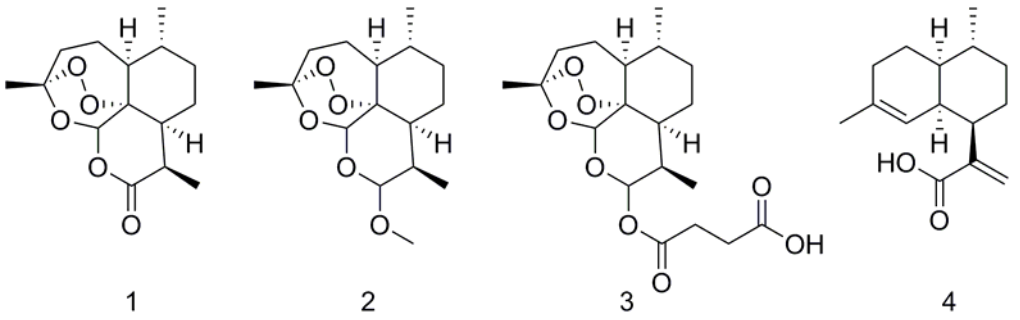
🕮 DEVELOPING ARTEMISIA ANNUA FOR THE EXTRACTION OF ARTEMISININ TO TREAT MULTI-DRUG RESISTANT MALARIA

Randolph R. J. Arroo [1*], Christopher J. Atkinson[2], Steven Bentley[3], Corrinne Burns[1], Michael J. Davies[2], Nigel Dungey[4], Ian Flockart[5], Colin Hill[6], Trevor Robinson[7], Lydia M. J. Smith[3], Jack G. Woolley[1]

[1] De Montfort University – Leicester School of Pharmacy, Leicester, UK, [2] East Malling Research, East Malling, UK, [3] National Institute of Agricultural Botany (NIAB), Cambridge, UK, [4] Humber VHB- Pharma Unit, Chicester, UK ,[5] Botanical Developments Ltd, Yalding, UK, [6] Extraction Technology Developments Ltd, Ashford, UK, [7] Frontier Agriculture Ltd, Lincoln, UK
e-mail: rrjarroo@dmu.ac.uk

Semi-synthetic derivatives of the sesquiterpene artemisinin (**1**) have worldwide become the main treatment for *P. falciparum* malaria. Artemisinin-combination therapies (ACTs), containing artemether (**2**) or artesunate (**3**) combined with non-isoprenoid drugs, are recommended as first line treatment by the World Health Organization, particularly in areas where resistance against quinine and quinine analogues has developed.

Whereas methods for the total synthesis of artemisinin have been developed[1], artemisinin extracted from the leaves of *Artemisia annua* L. (Asteraceae) is still the preferred source for commercial production of antimalarial drugs.



The biosynthetic pathway of artemisinin is well-known and a number of genes that regulate artemisinin biosynthesis have been identified. Various attempts have been made to enhance the yield of artemisinin in crops or plant cell cultures through the use of genetic engineering[2, 3]. Another approach has been semi-synthesis of artemisinin via artemisinic acid (**4**) in genetically engineered yeast[4].

Although genetic engineering holds a great promise for the future, currently the largest improvements in artemisinin yield have been obtained through creation of high-yielding varieties by classical breeding programs combined with modern agricultural production techniques[5].

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Part of the presented work was funded by the British Department for Environment Food & Rural Affairs through the Renewable Materials LINK Programme (LK0822)

🕮 SOLVOLYSIS OF 14,17-ETHENO-BRIDGED 17-ACETOXY-16-NITROSTEROIDS: MECHANISM AND SYNTHETIC APPLICATIONS

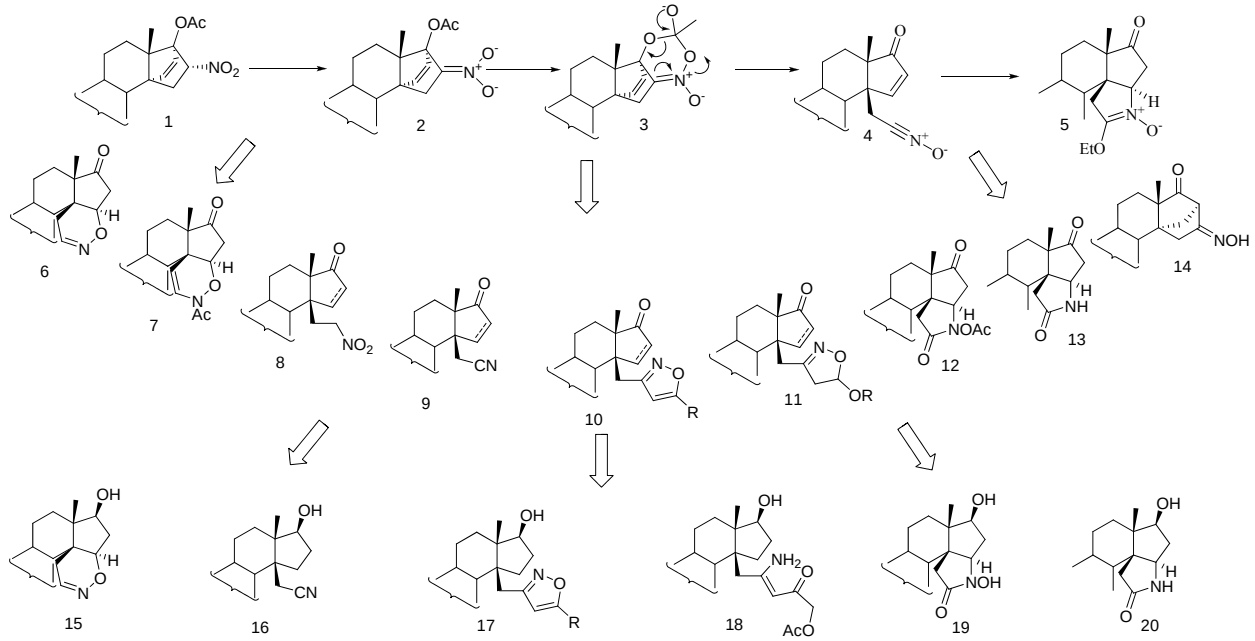
Alexander Baranovsky

Institute of Bioorganic Chemistry, National Academy of Sciences, Minsk, Belarus
e-mail: baranovsky@iboch.bas-net.by

The 14,17-etheno(ethano)-bridge, 17-acetoxy- and 16-nitro groups in steroid molecule form a very reactive fragment which allows preparing a wide variety of nitrogen containing heterocycles as well as 14β-chain products. The access to compounds **6-14** is based on one-step procedures employing compound **1** and its 17^1,17^2-dihydro analog. The attractive features of these procedures are their simplicity of execution, selectivity and a high yield in most cases.

The pathways leading to the mentioned compounds have been studied. They are separated in three types: the sigmatropic rearrangement (**6**), the retro-Henry reaction (**8**) and the nitrile oxide generation mechanism (**9-14**)[1,2]. The nitrile oxide formation in mild basic conditions ($NaHCO_3$, EtOH, H_2O, Δ) proceeds via intermediates **2** and **3**. Nitrile oxide **4** then can be trapped by a reducing agent (**9**), dipolarophile (**10**, **11**) or solvent (**5**, **13**).

The synthesized compounds were converted to D-ring substituted estradiol analogs (**15-20**) in order to test their biological properties.



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🕮 NEUROACTIVE STEROIDS AND VITAMIN D

Marie Bičíková

Institute of Endocrinology Prague , Czech Republic
e-mail: mbicikova@endo.cz

Neuroactive steroids (NAS) and neurosteroids have rapid, non-genomic actions in CNS. Changed NAS/neurosteroids levels are involved in many pathological processes. Regulation of their metabolism and understanding of the mechanism of actions will help to use them therapeutically.

The effects of these steroids are mediated mainly by modulation of ionotropic receptors of γ-aminobutyric acid ($GABA_{A)}$ or N-methyl-D-aspartate (NMDA) and so influencing influx of Cl^- or Ca^{2+} ions into the cells, resp. Among the best studied NAS belongs reduced progesterone derivative allopregnanolone – a positive modulator of $GABA_A$ receptors, with effects similar to those of benzodiazepines. On the other side, there are actually often studied or used dehydroepiadrosterone (DHEA) and its sulphate with their negative or positive modulation of $GABA_A$ or NMDA receptors, respectively.

NAS/neurosteroids have neuroprotective properties and participate in neuromodulation. Both the genomic and non-genomic effects of steroids in the brain may contribute to the pathophysiology of psychiatric disorders and the mechanisms of action of antidepressants or antipsychotics. The disorders in neurosteroid metabolome have been demonstrated in several neuro-psychiatric diseases (e.g. schizophrenia, Alzheimer disease, multiple sclerosis, depression etc.).

In the last few years as one of NAS is considered active form of vitamin D (calcitriol; 1,25dihydroxy-cholecalciferol). More and more communications are dealing with the relationship between vitamin D and psychiatric and neurological disorders. Neuroprotective role of vitamin D is explained e.g. by the reduction of reactive oxygen species (ROS) down-regulation of L-type of calcium channels or the expression of inducible nitric oxide synthase and support for the creation of glutathione. The growing number of contributions concerns the irreplaceable role of vitamin D in gravidity. Animal models show, that prenatal vitamin D-deficiency leads to alterations in fetal brain morphology and genes related to neuronal survival, speech and language development, and dopamine synthesis. Low saturation pre- and perinatal vitamin D may be a candidate risk factor for the later development of multiple diseases: multiple sclerosis, depression, seasonal affective disorders, schizophrenia, Alzheimer disease, Parkinson´s disease, autism, hypertension, diabetes mellitus 1, metabolic syndrome, osteoporosis, osteoarthritis and cancer. *In vivo* studies demonstrated that deficiency of vitamin D_3 may alter the adult brain with possibility of adverse neuropsychiatric implications.

The findings of more than two hundreds authors demonstrate that vitamin D supplementation might have a protective effect against above named diseases and that normalization of low serum vitamin D levels might contribute to the prevention of various neuropsychiatric disorders.

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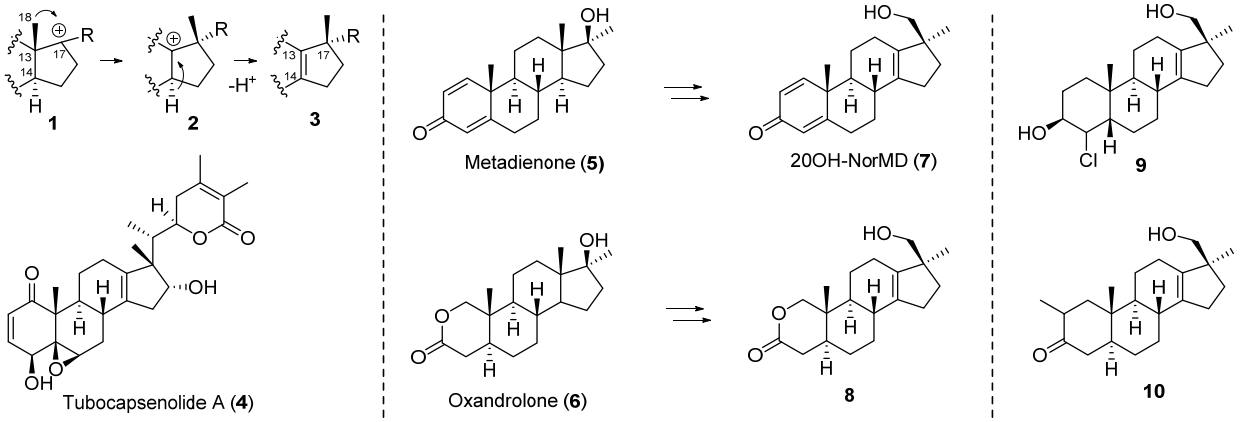
🕮 SYNTHESIS OF THE LONG-TERM METABOLITES OF ANABOLIC STEROIDS WITH A Δ^{13}-17α-METHYL-17β-HYDROXYMETHYL FRAGMENT

Alaksiej L. Hurski,* Maryia V. Barysevich, Tatsiana S. Dalidovich, Marharyta V. Iskryk, Nastassia U. Kolasava, Vladimir N. Zhabinskii, and Vladimir A. Khripach

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus
e-mail: AHurski@iboch.bas-net.by

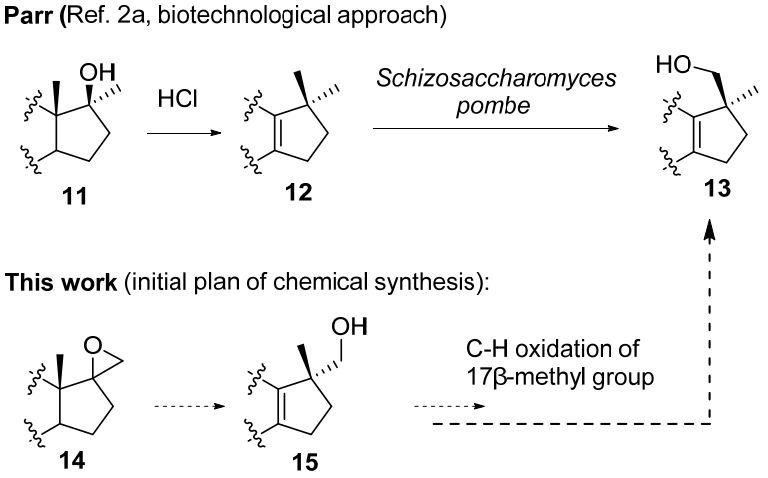
Steroidal 18-nor-13-ene fragment **3** arises as a result of a Wagner-Meerwein shift of the 18-methyl group to the C-17 cationic center of intermediate **1**, followed by deprotonation at C-14 (Scheme 1). The shifted methyl group in compounds produced in living organisms may remain non-functionalized as in cytotoxic steroid tubocapsinolide A (**4**) and some other withanolides,[1] or it may be hydroxylated as in the case of **7** (20OH-NorMD) and **8**. The latter compounds were found in the urine of athletes abusing performance-enhancing drugs metandienone (**5**)[2] and oxandrolone (**6**)[3] and these metabolites are used in doping control assays as reference substances. The benefit of **7** and **8** is that they can be detected for a much longer period of time (up to 19 and 16 days, respectively) after the administration of the parent anabolic androgenic steroids (AAS) as compared to other metabolites of **5** and **6**. The biotransformation studies of prohibited in sport AAS dehydrochloromethyltestosterone[4] and oxymetholone[5] resulted in identification of new long-term metabolites with the proposed structures **9** and **10** which also contain the 17β-hydroxymethyl-17α-methyl-18-norandrost-13-ene moiety. Because of a very small content of such metabolites in biological fluids, only mass-spectrometry and synthetic methods can be applied for their structure elucidation. Relatively simple structure of metabolite **7** was determined during investigation of metabolism of the deuterium-labeled metadienone (**5**) using mass-spectrometric data,[2b] but structures of more complex metabolites **9** and **10** have not been completely elucidated to date.[4-5] In this respect, synthesis of such AAS metabolites is needed both to confirm their proposed structures and for their application as reference substances in doping control assays.

From a synthetic point of view, the presence of a 17β-hydroxymethyl group in combination with a C13-C14 double bond in the D-fragment **13** of compounds **7**-**10** makes them challenging synthetic targets. To date, the only successful route to such compounds has been the biotechnological-based approach. It involves the hydroxylation of 17,17-dimethyl derivative **12** using CYP21 expressing recombinant strain CAD75 of fission yeast *Schizosaccharomyces pombe* for the preparation of 20OH-NorMD **7** from metandienone **5**[2a] or the fungus *Cunninghamella elegans* for the transformation of oxandrolone **6** into the metabolite **8** (Scheme 2).[3] Though having evident advantages in terms of the amount of steps to achieve the needed transformation, biotechnological approaches suffer from a lack of generality.



Scheme 1

In this keynote lecture, preparation of steroids **7** and **9** by chemical transformations will be reported. The proposed strategy to obtain **13** is based on a selective functionalization of unactivated C-H bonds[6] of the shifted 17β-methyl group in **15**. To achieve the required transformation, the potential of Pd-catalyzed acetoxylation assisted by a directing group[7] was used. The carbon backbone of the target fragment was built through a Lewis acid catalyzed Wagner-Meerwein rearrangement of readily available epoxide **14**.[8]



Scheme 2

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🕮 EFFECT OF BRASSINOSTEROIDS ON ION CHANNELS AND SIGNALLING IN ROOTS OF HIGHER PLANTS

Darya Straltsova[1], Palina Chykun[1], and Vadim Demidchik[1*]

[1]Belarusian State University, Minsk
e-mail: dzemidchyk@bsu.by

Brassinosteroids (BRs) are class of steroid hormones essential for the proper regulation of multiple physiological processes required for normal plant growth and development. Exogenous BRs can improve the quantity and quality of crops and ameliorates effects of stresses. Using native and synthetic analogues of BRs as a tool to improve plant yield seems to have a great potential for agriculture and biotechnology (Khripach, 2000). BRs have been intensively investigated for their biosynthesis, distribution and physiological functions using classical physiological tests, analyses of mutants and transgenic plants (*Arabidopsis thaliana* plants constitutively expressing aequorin). Recent data indicate that BRs are also sensed by the plasma membrane system catalyzing increase in the cytosolic free Ca^{2+} (in leaves of *Arabidopsis thaliana*). Zhao *et al*. (2013) have shown that the BR-induced elevation in the cytosolic free Ca^{2+} is abolished in knockout line lacking functional brassinosteroid receptor and after treatment with Gd^{3+} (blocker of Ca^{2+}-permeable nonselective cation channels) (Zhao, 2013). Zhang *et al*. (2005) using suspension culture cells of *Arabidopsis* have found that anion channel currents were inhibited by both 28-homobrassionolide and 28-castasterone and outwardly-directed K^+ conductance was stimulated by 28-homobrassionolide but inhibited by 28-castasterone (Zhang, 2005).

This study was to examine possible effects of brassinosteroids on the plasma membrane cation conductances in plant cells and related Ca^{2+} driven signalling events. Standard patch-clamp and aequorin chemiluminometry techniques were used (Shabala, 2006).

Here, we report the first electrophysiological characterisation of brassinosteroid-activated Ca^{2+}-permeable channels in higher plants. Wheat root protoplasts (tested by patch-clamping) and whole arabidopsis plants expressing Ca^{2+}-reporting protein, aequorin (analysed by chemiluminometry), were used in this study.

In the whole-cell patches (wheat root protoplasts), 1 μM 24-epibrassonolide, 28-homobrassionolide or 24-epicastasterone were applied exogenously. Only 24-epicastasterone modified transmembrane cation currents while 24-epibrassonolide and 28-homobrassionolide did not cause any reaction. Addition of 24-epicastasterone at cytosolic side through the patch-clamp pipette increased Ca^{2+} influx conductance, which demonstrated characteristics of depolarisation-activated Ca^{2+} channels. The pharmacological analyses have shown that brassinosteroid-activated Ca^{2+}-influx conductance was sensitive to inhibitors of Ca^{2+}-permeable cation channels. Blockers of K^+ channels did not inhibit this conductance.The plasma membrane conductance, which was activated by an endogenous 24-epicastasterone, showed bell-like shape with maximal activation at depolarisation voltages (bath: 20 mM Ca^{2+}). Labelling castasterone (and its derivates) with BODIPY (using castasterone-BODIPY conjugates which were synthesised chemically) showed that castasterone (and its derivates) can be transferred to the cytosol both in intact roots and protoplasts. This confirms that the effect of 24-epicastasterone at the cytosolic face can potentially be observed in real plants.

We also tested the effect of different brassinosteroids on cytosolic free Ca^{2+}, using *Arabidopsis thaliana* plants constitutively expressing aequorin. Brassinolide and castasterone, and its derivates (24-epibrassonolide, 28-homobrassionolide, 24-epicastasterone, 28-homocastasterone) were tested. All six brassionosteroids induced elevation of the cytosolic free Ca^{2+} in arabidopsis root cells. In the present study we demonstrated that 24-epicastasterone being more potent than 24-epibrassonolide and 28-homobrassionolide. 10 μM of exogenous BRs was the minimal concentration at which statistically significant changes of the cytosolic Ca^{2+} were observed.

The obtained results suggest that the plasma membrane of root cells contains the brassinosteroid-activated cation-permeable channels, which can be involved in cell ion homeostasis and signalling.

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🕮 PHYTOSTEROL BIOCONVERSION AS A PLATFORM FOR PRODUCTION OF VALUABLE STEROIDS: NOVEL FINDINGS AND PROSPECTS

Marina Donova*, Dmitry Dovbnya, Galina Sukhodolskaya, Sergei Khomutov, Andrei Shutov, Eugeny Bragin, Tanya Ivashina, and Nicolai Strizhov

G. K. Skryabin Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, 142290, Russia
*e-mail: *donova@ibpm.pushchino.ru*

Phytosterols are recognized now as most attractive, available and low-cost raw materials for steroid pharmaceutical industry. Phytosterols are produced in huge amounts from plants, such as soya, pine, or wastes of cellulose production plants. Their microbial transformation is an effective tool for the production of high-valued steroidal drugs and their precursors. Although a range of biocatalytic methods has been developed, selection of suitable microorganisms, as well as creation of new engineered strains is of great importance for generation of improved bioprocesses and production processes for obtaining known and new metabolites with potent biological activity. The achievements in genetic and metabolic engineering of steroid-transforming strains in combination with novel approaches in the enzymatic and whole-cell biocatalysis provide a platform for highly effective and selective biotransformations.

Actinobacteria are known to catabolize phytosterol via the 9(10)-secosteroid pathway.[1] Along with degradation of the aliphatic side chain, different modifications of steroid core occur during sterol bioconversion, such as 3β-hydroxy-5-ene to 3-keto-4-ene moiety transformation, Δ^1-dehydrogenation, 9α-hydroxylation. Metabolic blocks allow production of valued steroids by exploiting of the cascade reactions which are the part of the degradative pathway.[2]

Biotransformation of phytosterols by actinobacteria, and especially, by the selected strains of *Mycobacterium neoaurum* VKM Ac-1815D, 1816D and 1817D provides effective production of androstenedione (AD), androstadienedione (ADD) and 9α-hydroxyandrostenedione (9-OH-AD), respectively. These androstane steroids are the key intermediates in the synthesis of various steroid drugs.

Based on the whole genome sequencing, as well as on transcriptomic profiling, the specific genes and gene clusters had been revealed which are essential for specific steroid modifications.[3,4] The data were applied for the generation of engineered strains with improved biocatalytic possibilities for production of AD, 20-hydroxymethyl pregn-4-ene-3-one (20-HMP, BA) and their analogs. The knock-out of *fadD3* gene in *Mycobacterium smegmatis* mc2 155 allowed effective production of 4a-hydroxy-6a-methyldecahydro-cyclopenta[f]chromen-7(8H)-one (HMDC) in a single step from sterols. The recombinant strains capable of single-step converting of phytosterol, or cholesterol to testosterone, 1-dehydrotestosterone, progesterone were generated using heterologous expression of eukaryotic steroidogenic systems in mycobacterial hosts. Consequent bioconversions of phytosterol with two microbial strains, - *Mycobacterium neoaurum* VKM Ac-1815D and *Aspergillus ochraceus* VKM F-830, in one bioreactor vessel enable effective production of 11α-hydroxy-AD which is a key precursor in the syntheses of halogenated corticoids. Effective production of dehydroepiandrosterone (DHEA) from phytosterol has been provided by the combination of the protection-deprotection of the oxygen functionality at C3 with selective side chain degradation of the 3-substituted sterols using *Mycobacterium neoaurum* VKM Ac-1815D. Re-

gio- and stereospecific hydroxylation of DHEA at positions 7α, 7β and double hydroxylation at 7α,15α using selected fungal strains provide effective production of the valued 3β-ol-5-ene derivatives.[5]

Effective procedures for the isolation and purification have been developed which provided high purity of the final crystalline products.

Thus, exploiting of mycobacterial phytosterol catabolic pathway at the generation of engineered strains allows of improved bioprocesses and effective production schemes for obtaining of a whole number of the high-value steroids (Fig. 1).

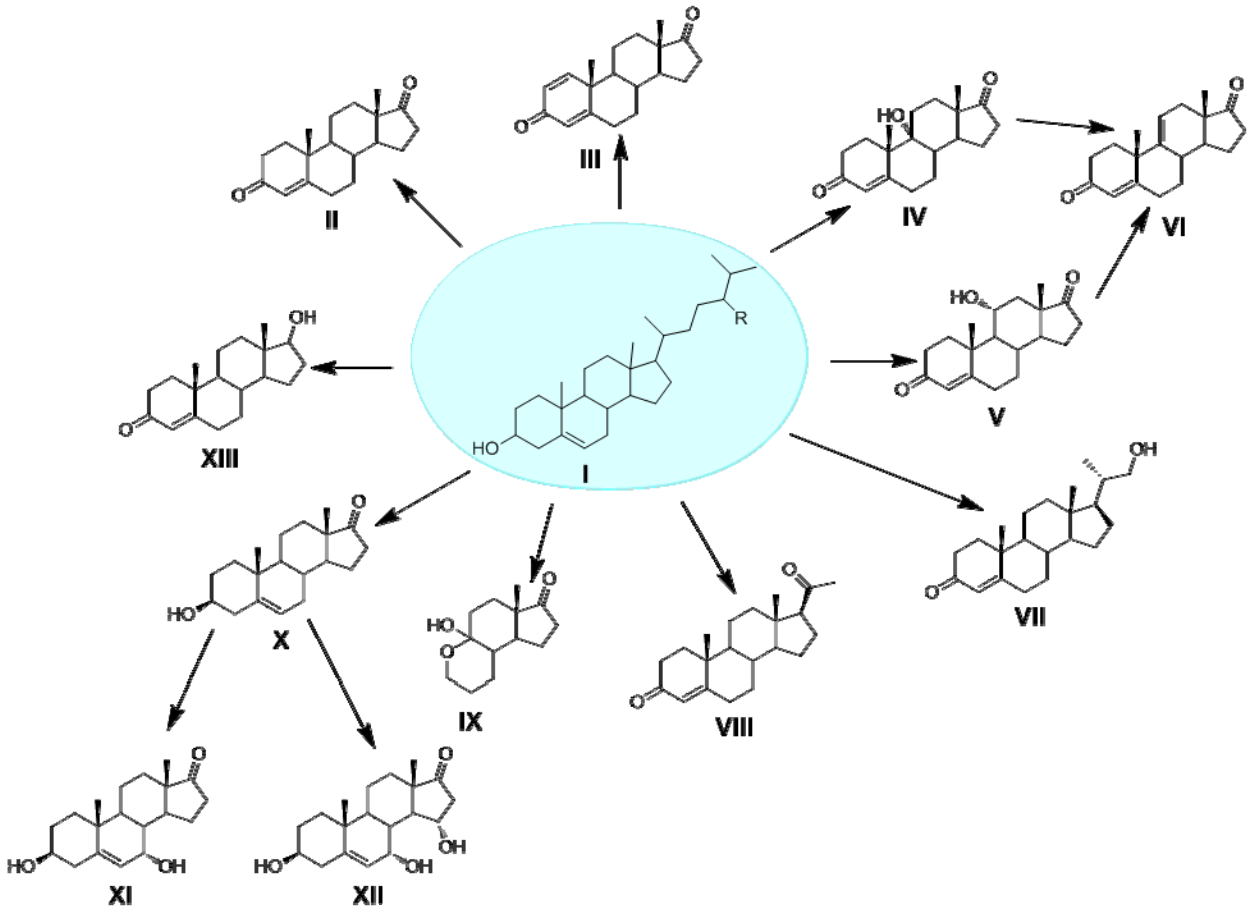

Figure 1. Valuable steroids produced from phytosterol (I) using bioconversion methods: II – androst-4-ene-3,17-dione (AD); III – androsta-1,4-diene-3,17-dione (ADD); IV - 9α-hydroxy-AD; V - 11α-hydroxy-AD; VI – androst-4,9(11)-diene-3,17-dione (Δ9(11)-AD); VII – 20-hydroxymethylpregn-4-ene-3-one (20-HMP, BA); VIII – progesterone; IX - 4a-hydroxy-6a-methyldecahydro-cyclopenta[f]chromen-7(8H)-one (HMDC); X – 3β-hydroxyandrost-5-en-17-one dehydroepiandrosterone (androstenolone, prasterone, DHEA); XI - 3β,7α-dihydroxyandrost-5-ene-17-one (7α-hydroxy-DHEA); XII - 3β,7α,15α-trihydroxyandrost-5-ene-17-one (7α,15α-dihydroxy-DHEA); XIII - androst-4-ene-3-one-17β-ol, testosterone

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🕮 THE EFFECT OF BRASSINOSTEROIDS ON SIGNALING SYSTEMS AND METABOLISM REGULATION IN PLANT CELLS UNDER SALT STRESS

Volodymyr Kravets[a]*, Michael Derevyanchuk[a], Sergii Kretynin[a], Raisa P. Litvinovskaya[d], Jan Martinec[b], Yaroslav Blume[c], and Vladimir A. Khripach[d]

[a]Department of the Molecular Mechanisms of Cell Metabolism Regulation, Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine, 02660, Murmanska str., 1, Kyiv, Ukraine, [b]Department of Biology, Faculty of Sciences, Jan Evangelista Purkyne University, Usti nad Labem, Czech Republic, [c]Institute of Food Biotechnology and Genomics, NAS of Ukraine, Osypovskogo 2a, Kiev 04123, Ukraine, [d]Laboratory of Steroid Chemistry, Institute of Bioorganic Chemistry, NAS of Belarus, 220141, Kuprevich str., 5, Minsk, Belarus
e-mail: kravets@bpci.kiev.ua

Brassinosteroids (BRs) are steroid plant hormones that act as key regulators of plant growth and stress tolerance. The effect of BRs on plant life cycle is initiated by hormone-activated complex signaling systems. The roles of its components in the control of cell metabolism under stress action are not disclosed yet.

The aim of this study was to investigate the influence of brassinosteroid, 24-epibrassinolide (24-EBR) on *Brassica napus* seedlings and *Arabidopsis thaliana* plants: wild-type (WT) *A.thaliana* plants and two transgenic lines – overexpressing alternative oxidase *AOX1a* (AOX1a-OE) and antisense line (AOX1a-AS) under control and salt stress conditions.

We have observed that 24-EBR stimulates formation of key lipid messengers - phosphatidic acid (PA) and diacylglycerol (DAG). Utilizing fluorescent and P^{33} labeled lipids and pharmacological approach with specific inhibitors to key enzymes involved in PA and DAG conversion we found that BRs activate phosphatidylcholine-hydrolyzing phospholipase C (NPC). PA level in mutant *bak1* plants was significantly decreased that indicates involvement of receptor kinase BAK1 in BR-induced activation of phospholipases.

It was investigated role of 24-EBR in facilitation of energy homeostasis in plants (activities of cyanide-sensitive and cyanide-resistant respiratory pathways, synthesis of molecular mitochondrial chaperones) and activation of antioxidant systems to prevent oxidative stress development. It was established that lowering level of endogenous BRs with biosynthetic inhibitor brassinazole suppressed alternative respiration under severe salinity conditions. Exogenously applied 24-EBR stimulated activity of cyanide-resistant respiratory pathway by 2-folds. Respiration rate of mutant *bri1* plants was not affected by exogenous 24-EBR. This is an evidence of receptor kinase BRI1-mediated regulation of plant respiration by BRs. It was observed that 24-EBR induces synthesis of probable molecular mitochondrial chaperones which may function to protect respiratory chain.

This work was supported by the SFFR of Ukraine (grant F70), NAS of Ukraine grants (9.1-16(06), №14-16).

🕮 NATURAL AND SEMISYNTHETIC SESQUITERPENE LACTONES IN CELLULAR CONTEXT: FOCUS ON LIVE-CELL IMAGING AND GENE EXPRESSION ANALYSIS

Silvie Rimpelová[1*], Michal Jurášek[2], Hynek Strnad[3], Vladimíra Pavlíčková[1], Lucie Peterková[1], Eva Kmoníčková[4], Tomáš Ruml[1], and Pavel Dražar[2*]

[1] Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Prague, Czech Republic, [2] Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Prague, Czech Republic, [3] Laboratory of Genomics and Bioinformatics, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [4] Institute of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University, Prague, Czech Republic
e-mail: silvie.rimpelova@vscht.cz; pavel.drasar@vscht.cz

Sesquiterpene lactones are bioactive natural compounds with high potential in various medicinal disciplines[1] employing of the plethora of their wide spectra of biological activities, such as one of the uppermost interest – anticancer properties. Among the other certainly belong antimicrobial, anti-inflammatory and antiparasitic activities, as well as effects on central nervous and cardiovascular systems.[2,3] Two well-known examples of this group of compounds are guaianolides thapsigargin and trilobolide, which are interesting mainly for their antiproliferative properties and immunostimulatory activity.[4,5] Another structurally related analog of these sesquiterpene lactones, archangelolide, has been yet nearly undescribed. Therefore, in our study, we have focused on revealing of differences among the biological properties of these compounds, as well as of their fluorescently labeled analogs enabling their tracking directly on the site of localization in living cells of various model cancer cell lines.[6] Cytotoxicity, metabolic activity and nitric oxide production of cells treated with these derivatives were also examined. Moreover, and most importantly, we have performed whole genome expression profiling using DNA microarray analysis based on Illumina HumanWG Expression BeadChips to further disclose the differences among these three sesquiterpene lactones. The aim was to identify the biological pathways affected by the individual compounds, and to compare their antiproliferative potential and genes affected in an *in vitro* model of human prostatic cancer.

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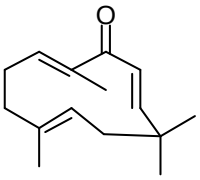
🕮 ZERUMBONE: THE FASCINATING CHEMISTRY OF A MACROCYCLIC SESQUITERPENOID

Giovanni Appendino[1*], Alberto Minassi[1], Giuseppina Chianese[2], Federica Pollastro[1], Eduardo Munoz[3], and Orazio Taglialatela-Scafati[2,*]

[1] Dipartimento di Scienze Del Farmaco, Università del Piemonte Orientale, Novara, Italy, [2]Dipartimento di Farmacia, Università di Napoli Federico II, Napoli, Italy, [3] Department of Cell Biology, Physiology and Immunology, University of Córdoba, Córdoba, Spain
e-mail: scatagli@unina.it

Zerumbone (**1**), a humulane sesquiterpenoid whose structure includes a 11-membered ring and a dienone system, is the major volatile constituent of *Zingiber zerumbet* (shampoo ginger), an Indian plant used both as food flavouring and medicine in India and the Far East.[1] Zerumbone (**1**) has been the object of a growing interest due to its "cure-all" bio-pharmacological profile, similar to that of curcumin, of which it could be considered a lipophilic version. Considering only the last couple of years, antiulcer and antihypercholesterolemic activities have been added to the largely documented anticancer potential.[2]



1 (Zerumbone)

The biological activity of zerumbone has been often related to the Michael acceptor behaviour of the cross-conjugated dienone system, although this group should be in principle poorly reactive. Examples of conjugated additions had already been reported, but we have recently investigated the possible thiol trapping activity of zerumbone through the cysteamine assay.[3] In this assay, the dienone system of zerumbone reacted with two equivalents of cysteamine to give a bis-adduct, with a marked difference in the reversibility behaviour. *E/Z* photochemical isomerization of zerumbone caused dramatic effect on the site, the mode (transient or irreversible), and the stoichiometry of the reaction.[4] Interestingly, we have also evidenced a substantial separation between Michael reactivity and biological activity toward endpoints sensitive to thiol trapping. This supports the view that shape complementarity plays a critical role in the covalent binding of Michael acceptors to their macromolecular target(s).

Finally, we have explored the reactivity of zerumbone (**1**) with acids, unveiling that a cornucopia of cyclized derivatives were produced by treatment with Lewis acids. Surprisingly, the course of the cyclization was dramatically dependant on the exact nature of the Lewis acid, and both carbocyclic and aromatic systems could be generated.

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🕮 ORAL COMMUNICATIONS

🕮 DISTRIBUTION IN THE NATURE OF SESQUITERPENE LACTONES WITH THE NON-TYPICAL STRUCTURE OF MOLECULES

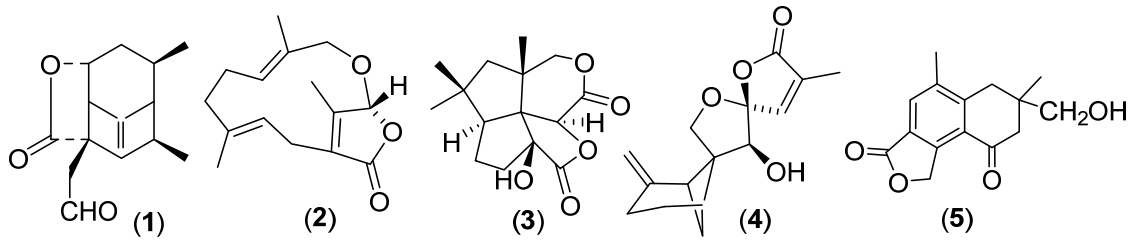
Sergazy M. Adekenov

International Research and Production Holding "Phytochemistry", Karaganda, Republic of Kazakhstan
e-mail: arglabin@phyto.kz

The improvement of methods for the isolation of natural compounds and the study of a structure of their molecules has revealed new sesquiterpene lactones with non-typical structural skeletons. Over the past 20 years (1995-2015) were isolated and described more than 2500 sesquiterpene lactones belonging to seven major structural types (guaiane , germacrane, lindenane, pseudoguaiane, eudesmane, elemane, eremophylane). The minor quantity in natural sources contains sesquiterpene lactones of such structural types as aromandendrane, aristolane, bisabolane, botridiane, valerane, illudane, carabrane, cuparane, oplopane, sterpurane, hirsutane and other rare and non-typical carbon skeletons.

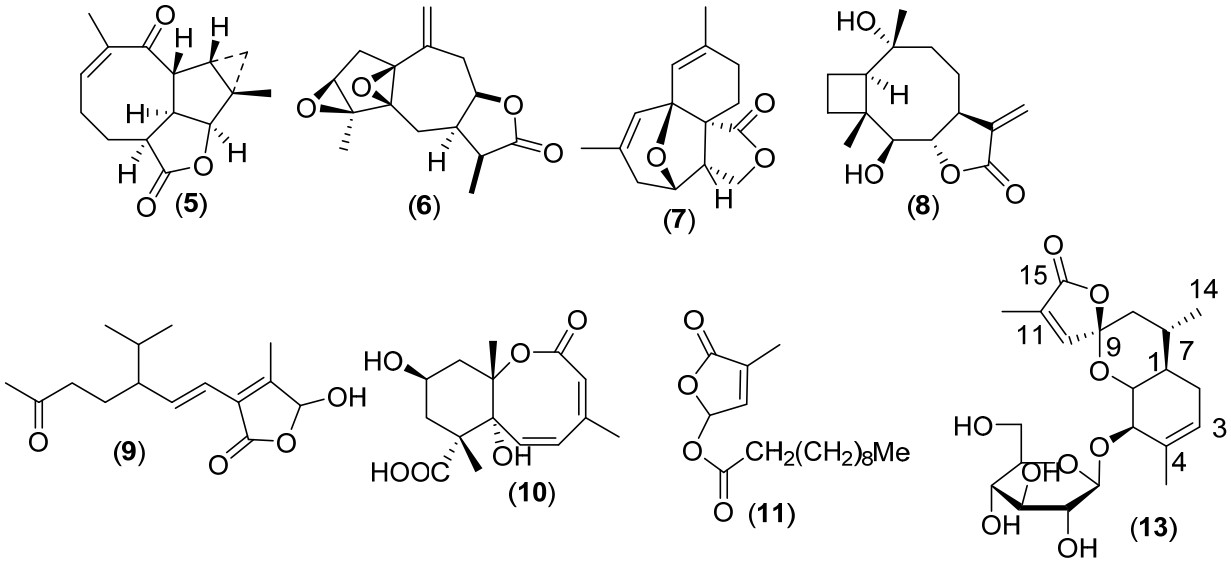
The majority of sesquiterpene lactones from marine origin characterized by furan, selinane, aristolane, lemnalane, chamigrane skeletal types with rare for natural compounds functional groups as isonitrile, isothiocyanate, formamide. Non-typical sesquiterpene lactones of marine metabolites formed in accordance with the biogenetic isoprene rule (farnesyl) and contradicting it (non- farnesyl). Several sesquiterpenoids of marine organisms have regrouped non-isoprenoid skeleton, for example, upial (**1**) of *Dysidea fragilis*. From marine plants identified a number of lactones with a new unique carbon skeleton, for example, menelloide A (**2**) from *Menella sp.,* asperaculine A (**3**) with a new [5,5,5,6] fenestrane ring from the marine fungus *Aspergillus aculeatus* CRI323-04, massarinoline A (**4**) c with rare ring system of marine fungi *Massarina tunicata*.

It is known about the isolation of the new terpene alcohol stenotarsol (**5**) from a beetle *Stenotarsus subtilis* (Endomychidae). This compound is a new type of terpene skeleton and is the first of the secondary metabolites isolated from family of beetle.



The greatest amount of sesquiterpene lactones with a new carbonic cycle is isolated from flowering plants - representatives of *Asteraceae* family, for example, non-typical 14,15-dimethyl-7,13-dioxotricycle [6.4.0.$0^{9,11}$]dodeca-12,13-olide (**6**) with functional groups, rare for natural molecules isolated from *Nauplius graveolens subsp. odorus*, (−)-1,5; 3,4-diepoxy-10(14)-en-12,8-olide (**7**) from two β-focused epoxy cycles in a five-membered carbocyclic ring from *Stevia tomentosa*, antecularine (**8**) of *Anthemis auriculata*, formed out of farnesyl pyrophosphate way, dimeric lactones from various species of this family. Sesquiterpene lactones of a unique structure have also been isolated from plants of other families, for example, michampanolide (**9**) with a new skeleton from *Michelia champaca*, sipandinolide (**10**) of *Siparuna andina*, amygdalactone (**11**) of *Prunus amygdalus*, litseabutenolide (**12**) of *Litsea verticillata*. Molecules with spiro-2 (5H)-furanone

fragment rarely isolated from natural sources especially with sesquiterpenoid skeleton. From the green mass of *Abies dalavayi* the new sesquiterpene lactone is isolated (**13**).



Thus, the majority of new sesquiterpene lactones with non-typical carbon skeletons contain in plant based raw materials, a significant amount of such lactones isolated from marine organisms, and relatively few can be found among secondary metabolites of microorganisms.

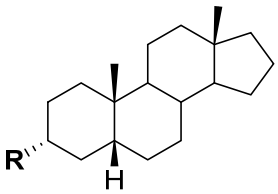
🕮 PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF NOVEL AMIDE-BASED STEROIDAL INHIBITORS OF NMDA RECEPTORS

Santosh Kumar Adla[1], Barbora Slavikova[1], Marketa Smidkova[1], Eva Tloustova[1], Martin Svoboda[1], Vojtech Vyklicky[2], Barbora Krausova[2], Pavla Hubalkova[2], Michaela Nekardova[1,3], Kristina Holubova[2], Karel Vales[2], Milos Budesinsky[1], Ladislav Vyklicky[2], Hana Chodounska[1], and Eva Kudova[1,*]

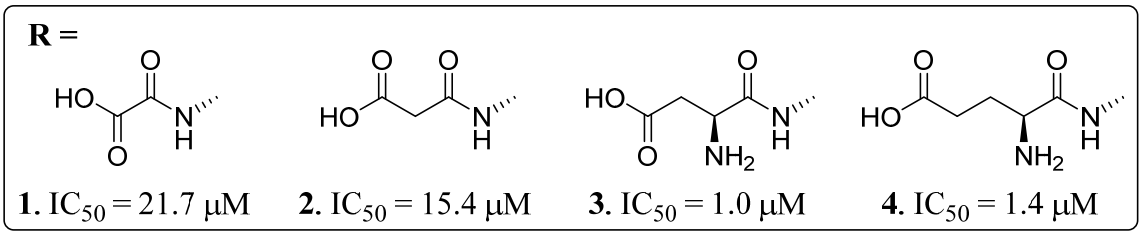
[1] Institute of Organic Chemistry and Biochemistry (IOCB), Academy of Sciences of the Czech Republic, v.v.i., Flemingovo nam. 2, Prague 6 – Dejvice, 16610, Czech Republic. [2] Institute of Physiology CAS, Videnska 1083, 142 20 Prague 4, Czech Republic , [3] Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 3, Prague 2, 121 16, Czech Republic.
e-mail: kudova@uochb.cas.cz,

Herein, we report a new class of amide-based inhibitors (**1**-**4**) of *N*-methyl-*D*-aspartate receptors (**NMDAR**s) that were prepared as analogues of pregnanolone sulfate (**PAS**) and pregnanolone glutamate (**PAG**) – the steroidal neuroprotective NMDAR inhibitors. A series of experiments were conducted to evaluate their physicochemical and biological properties: *(i)* the inhibitory effect of compounds **3** and **4** on NMDARs was significantly improved (IC_{50} = 1.0 and 1.4 μM, respectively) as compared with endogenous inhibitor – pregnanolone sulfate (IC_{50} = 24.6 μM) and pregnanolone glutamate (IC_{50} = 51.7 μM); *(ii)* physicochemical properties (logP and logD) were calculated; *(iii)* Caco-2 assay revealed that the permeability properties of compounds **2** and **4** are comparable with pregnanolone glutamate; *(iv)* compounds **1**-**4** cross blood-brain-barrier.

Amide-based inhibitors of NMDA receptors



- ☑ Strong inhibitors of NMDA currents
- ☑ Evaluated in Caco-2 Permeability Assay
- ☑ logP and logD values calculation
- ☑ Do cross blood-brain-barrier



1. IC_{50} = 21.7 μM **2**. IC_{50} = 15.4 μM **3**. IC_{50} = 1.0 μM **4**. IC_{50} = 1.4 μM

This work was supported by grants: (a) TE01020028 - Center for Development of Original Drugs from the Technology Agency of the Czech Republic, (b) 303/12/1464 - Grant Agency of the Czech Republic, (c) research project grant RVO 67985823, (d) NPU I - LO1302 - National Programme sustainability, Ministry of Education, and (e) 15-29370A - Ministry of Health of the Czech Republic.

🕮 SYNTHESIS AND PROPERTIES OF NEW DERIVATIVES OF MALEOPIMARIC AND CITRACONOPIMARIC ACIDS

Maksim P. Bei* **and Anatolij P. Yuvchenko**

The Institute of Chemistry of New Materials of NAS of Belarus, Minsk, Belarus, e-mail: beymaksim@gmail.com

One of the most promising directions of plant resources use is the preparation of fine chemicals. The terpenes from wood resins (essential oils and rosins) constitute one of the valuable parts of plant resources. Essential oils widely used for production of fine chemicals but diterpene part of wood resins (rosin) is used mostly for production of bulk chemicals (printing inks, adhesives, glues etc.). The most available diterpenoid substance − maleopimaric acid (MPA) that may be isolate from maleated rosin, serves as a valuable synthon for the preparation of a wide range of compounds. We developed some new efficient methods for synthesis of new maleopimaric acid derivatives with valuable properties − esters, amides, imides, diimides **1–5**:

– Previously unknown unsaturated esters of MPA **1** were prepared by reaction of MPA with allyl- and propargyl bromide in high yields (83–95%).[1]

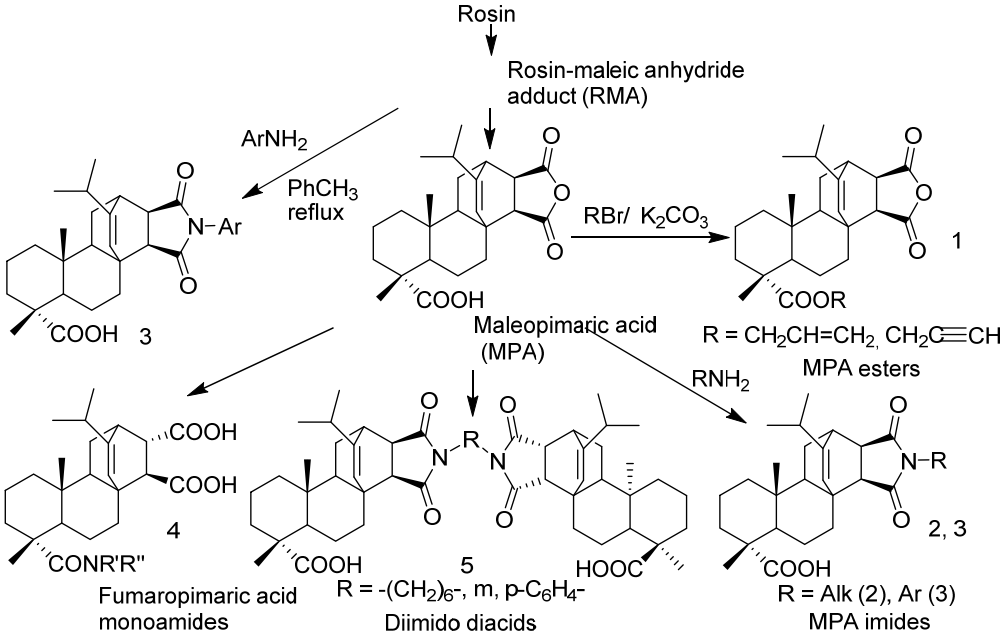
– Method of synthesis of maleopimaric acid *N*-(*n*-alkyl)imides **2** by the reaction of MPA with primary aliphatic amines (butyl-, hexyl-, octyl-, dodecyl-, octadecylamine) in melt was developed. This method allows to produce maleopimaric acid *N*-(*n*-alkyl)imides **2** with quantitative yields without applying excess of primary amines and organic solvents.[2]

– Method of synthesis of individual maleopimaric acid N-arylimides **3** from maleated rosin, without preliminary isolation of MPA. This method allows producing of pure maleopimaric acid N-arylimides with 46−80 mass. % yields (from started rosin).[3] In contrast to MPA aliphatic imides, MPA N-arylimides are high-melting (280−350^{0}C) compounds with low solubility in common organic solvents.

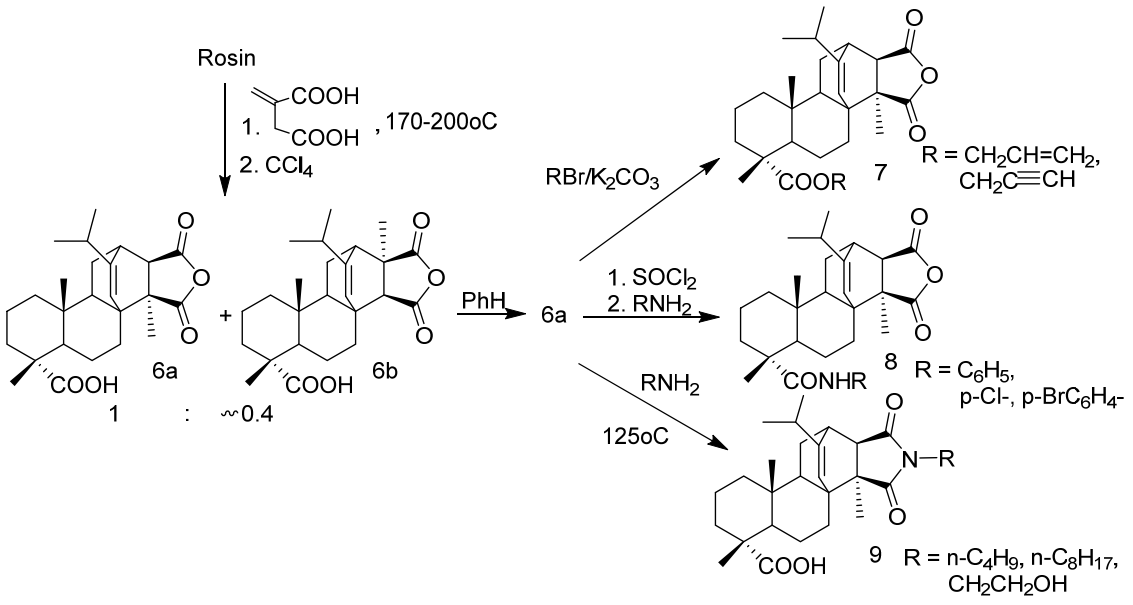
– Method of preparation of a new type of terpenoid compounds – monoamides of fumaropimaric acid **4**, with *trans*-1,2-dicarboxylic fragment in it's

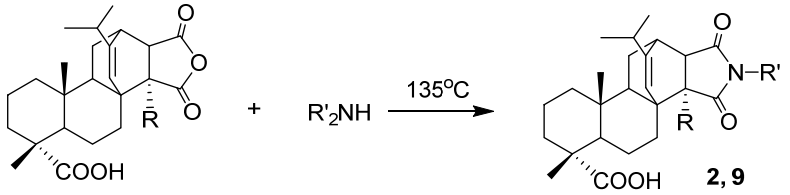
structure, by alkali isomerization of maleopimaric acid amides was developed.[4]

– Method of synthesis of diimido diacids **5** from MPA and hexamethylenediamine, *p,m*-phenylenediamines.[1,3]



Citraconopimaric acid (CPA, **6**) is an analog of MPA bearing methyl group at α-position of cyclic anhydride group. Previously, CPA was not isolated in individual state and described. Method of synthesis of isomer of citraconopimaric acid **6a** bearing methyl group at C-15 was developed.[5] The method includes preparation of the adduct of pine rosin with citraconic anhydride (formed *in situ* from itaconic acid) followed by recrystallization of the product from carbon tetrachloride. The precipitate contains two isomeric citraconopimaric acids **6a, b** poorly separable by chromatography methods, however, the isomer of CPA **6a** containing methyl group at C-15 was obtained in pure form by partial crystallization of the mixture. Previously unknown unsaturated allyl and propargyl esters **7**, amides **8** and imides **9** of CPA were prepared.[6]





Reaction of maleopimaric and citraconopimaric acids with some secondary amines (diethyl-, dipropyl- and dibutylamine) was investigated and the formation of N-substituted imides of citraconopimaric and maleopimaric acids **2, 9** instead of the expected amidoacids was found.[7] Only reaction of MPA with diethyl-, dipropylamine at 135ºC gave expected amidoacids in 10−15% yields together with MPA N-ethyl-, N-propylimides (60−80% yields).

It was shown that the synthesized compounds can be applied as additives to the industrial polymers, chiral additives to the nematic liquid crystals, biologically active compounds.[6,8]

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🕮 INTERACTION OF HUMAN STEROID 7α-HYDROXYLASES WITH BRASSINOSTEROIDS

Yaraslau V. Dzichenka and Sergey A. Usanov

Institute of Bioorganic chemistry of National Academy of Sciences, Minsk, Belarus
e-mail: dichenko@iboch.bas-net.by

Cytochromes P450 (CYP) are superfamily of widespread in nature heme-containing enzymes that catalyze transformation of multitude chemical substances [1]. Among them steroid 7α-hydrohylases CYP7A1, CYP7B1, CYP39A1 are remarkable by their key role in the process of bile acids biosynthesis from cholesterol [2].

Brassinosteroids (BS) are steroid class phytohormones that support normal functioning of plants mostly in the processes of growth and adaptation to adverse conditions [3]. According to the accumulated wide experimental material BS also possess their activity not only in plants but also in the organisms of other kingdoms. In particular, it was shown that they have anticholesterolemic effect [3], but exact mechanism of their action in this case as well as in the majority of other cases is still unknown.

In the present work interactions of human steroid 7α-hydrohylases with brassinolide, epibrassinolide and epicastasterone were investigated. It was established that among the examined enzymes only CYP7A1 is capable to bind epibrassinolide.

Using molecular docking method, it was discovered that the molecule of the ligand stands in CYP7A1 active site similarly to cholesterol molecule. Moreover, it was shown that in stabilization of epibrassinolide take part amino acid residues which are principal for substrate molecules binding [4]. This fact denotes the possibility of hydroxylation of epibrassinolide by CYP7A1 at 7 position.

Thereby it was shown that human cholesterol 7α-hydrohylase participates in epibrassinolide transformation. Establishment of the structure of the generated product and its role in organism functioning is the aim of further work.

The results obtained contribute to the understanding of molecular mechanism of brassinosteroids action on human organism.

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🕮 IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS ENZYMES INVOLVED IN METABOLISM OF IMMUNOACTIVE STEROLS

Andrei A. Gilep[1], Anna V. Vasilevskaya[1], Tatyana V. Shkel[1], Sergey A. Usanov[1], Irina P. Grabovec[1], Valeria A. Kopot[1], Alexander P. Lysenko[2], Artyom P. Lemish[2], Gennadiy V. Sergeev[1], Dmitry O. Dormeshkin[1], Sviatlana V. Smolskaya[1], and Natallia V. Strushkevich[1]

[1]Institute of Bioorganic Chemistry, NASB, 220141, Minsk, Kuprevicha 5/2, Belarus; [2] Institute of an Experimental Veterinary Science NASB, 220003, Minsk, Briketa 28, Belarus
e-mail: agilep@yahoo.com

Introduction: Problems associated with treatment of XDR forms of tuberculosis are well known. Identification of new molecular drug targets is of particular importance to combat the disease. Members of the cytochrome P450 family (CYP) considered as potential candidates being involved in metabolism of biologically important molecules in the host organism. *Mycobacterium tuberculosis* has unusually high number of different cytochrome P450s (20 genes). Despite intensive research in this field 75% of these enzymes remain orphans. Our research is focused on investigation of mycobacterial P450 that might be involved in metabolism of bioactive sterols.

Materials and Methods: We apply protein family based approach in our experiments to study the panel of CYPs using methods of biotechnology, enzymology, structural biology, bioinformatics as well as experimental and technological practices in synthesis of compounds with desired properties.

Results: In present work we carried out molecular cloning, heterologous expression and purification of 8 cytochrome P450s from XDR strains of *Mycobacterium tuberculosis*. The substrate specificity of these enzymes was studied using various steroid and vitamin D derivatives. We identified mycobacterial cytochrome P450 that shows hydroxylation activity toward vitamin D and provitamin D3 in vitro. We also found that mycobacterial P450s can metabolize bioactive oxysterols.

Conclusions: We have discovered that pathogenic mycobacteria are involved in metabolism of immunoactive sterols. Based on these results we suggest that modulation of oxysterol and secosteroid biosynthesis or metabolism could be an adaptation mechanism of mycobacteria infection for human immune system.

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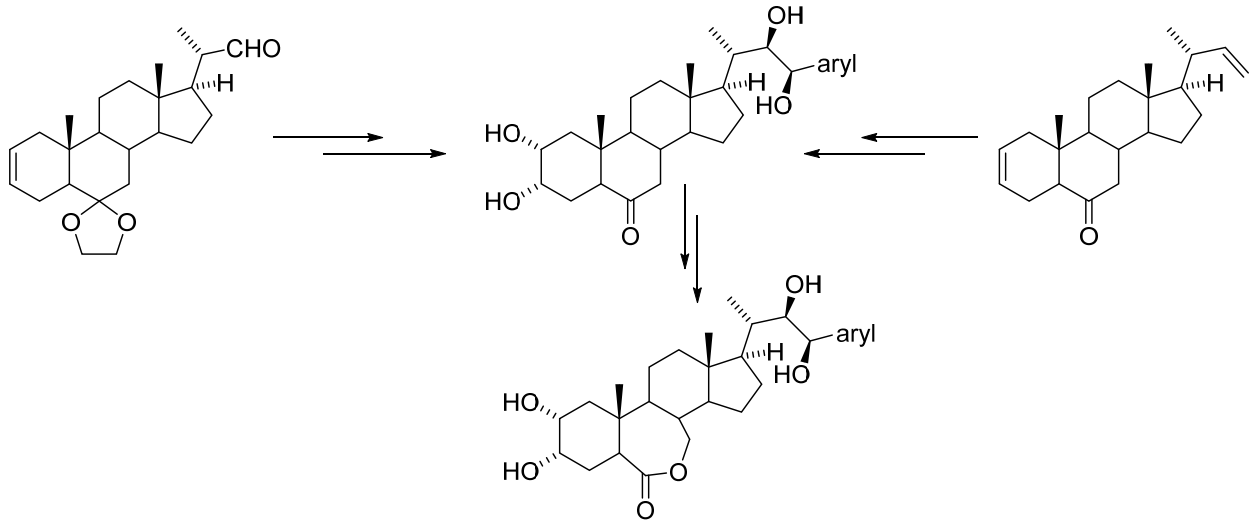
🕮 DESIGN AND SYNTHESIS OF NOVEL ARYL ANALOGUES OF BRASSINOSTEROIDS

Miroslav Kvasnica,* Petra Kořínková, Jana Oklestkova, Jaromír Mikulík, Václav Bazgier, and Miroslav Strnad

Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany CAS & Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic.
e-mail: miroslav.kvasnica@ueb.cas.cz

Brassinosteroids (BRs) are steroid plant hormones essential for many aspects of plant growth and development, like cell division, elongation and differentiation, pollen tube growth, seed germination, regulation of gene expression, enzyme activation and photosynthesis.[1] They are also involved in defense against a wide range of biotic and abiotic stresses. In contrast with mammalian nuclear steroid receptor, BRs are perceived at the cell surface by the transmembrane receptor complex formed by the receptor kinase BRI1 and its co-receptor BAK1.[2] BRI1 receptor has binding site for BRs located in extracellular ectodomain. There, the nonpolar side of BRs fit into a highly nonpolar cavity and hydroxyl groups of BRs are exposed to the solvent or towards interaction with BAK1. This information leads us to the idea of modification brassinosteroid tail using some nonpolar groups like phenyl.

We prepared a series of new brassinosteroid derivatives with *p*-substituted phenyl group in the side chain (Scheme 1). Phenyl or substituted phenyl groups were chosen based on successful molecular docking into the active site of BRI1 using AutoDock Vina. Some compounds showed promisingly very strong interaction with BRI1 receptor. Two basic approaches were chosen for preparation of these analogues. They were based on Horner-Wadsworth-Emmons reaction and metathesis.



Scheme 1: General scheme for synthesis of 23-aryl analogues of brassinosteroids.

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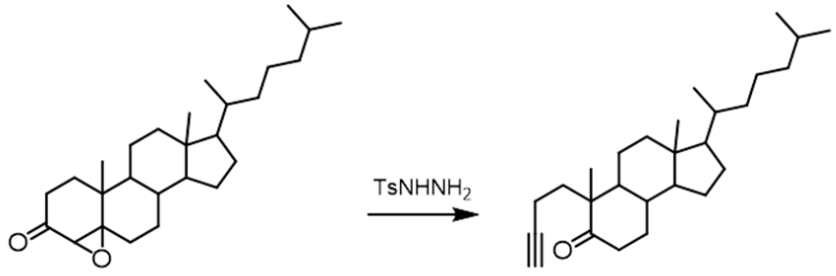
🕮 RING-A-OPENED STEROID: MEMBRANE ALKYNE LABELING ENABLING EFFECTIVE RAMAN IMAGING, CLICK CHEMISTRY AND ON-SITE CHEMICAL ACTIVATION

Satoshi Yamaguchi[1], Taku Matsushita[2], and Akimitsu Okamoto[1,2*]

[1]Research Center for Advanced Science and Technology (RCAST), The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo, Japan, [2]Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
e-mail: okamoto@chembio.t.u-tokyo.ac.jp

Intracellular distribution and metabolism of steroids are closely related to important biological events and diseases. The methods for monitoring the dynamic changes in the intracellular localization of steroids have been extensively studied: cholesterol have been mainly visualized by using cholesterol-binding molecules and cholesterol analogs. In most cases, these reporter molecules were labelled with fluorescent dyes and observed by fluorescent microscopy. Alkynes have also recently been attracting attention as a label to replace the bulky charged fluorescent dyes. The size of alkynes is much smaller and they do not induce any intermolecular electrostatic interaction. In addition, carbon−carbon triple bonds in alkyne compounds work effectively for not only Huisgen cycloaddition but also Raman imaging. However, as can be seen from the fact that the compounds containing carbon−carbon triple bonds exist very rarely in cells, it would be very difficult to obtain a carbon−carbon triple bond from its precursor under physiological conditions by use of external stimuli. The stimuli-responsive probe has been reported to be one of promising tools for clearly visualizing the dynamic distribution changes even in high background signals. However, there is still no alkyne steroid probe activated by external stimuli, although such an activatable alkyne steroid probe would be a powerful signal molecule for cell-specific Raman imaging assays.

We first designed an alkyne-labeled steroid. When steroids insert into lipid bilayer membrane, the ring D tethering an alkyl chain locates at the deep inside of the membrane, accordingly the ring A directs to the membrane surface. If we need any modification for the cholesterol after insertion to membrane, the ring A located close to the membrane surface would be a top candidate structure for introduction of an easily accessible reaction point into cholesterol. Therefore, we designed the alkynyl probe **2**, which is the compound that the ring A of cholesterol is replaced with an alkynyl group. The probe **2** was efficiently synthesized through epoxidation and subsequent Eschenmoser−Tanabe fragmentation from 4-cholesten-3-one.



The probe **2** may work like natural cholesterols on the membrane. To evaluate the behavior analogy between cholesterol and **2**, their abilities of lipid domain formation on liposomes were examined using fluorescence imaging. On the giant liposome consisting of dioleylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC) and **2** (40:40:20), the raft-like domains were stained with fluorescein-labeled Cholera toxin subunit B. The other membrane domain than the raft-like domain was fluorescently stained with Rhodamine DHPE. The giant liposome was also prepared by employing cholesterol instead of **2**, followed by staining with the two fluorescent probes. On the DOPC/DPPC/**2** system, the two domains which were separately stained with each fluorescent

probe were formed, sugessting that **2** has the ability to form the raft-like domain as observed in a cholesterol system.

The alkyne **2** has a characteristic Raman signal and applicable to alkyne-tag Raman imaging, which can specifically visualize the target molecules without great influence on their intrinsic properties because of the small size of alkyne-tag. The alkyne **2** on the lipid bilayer membranes was visualized through mapping the Raman signal at 2100 cm^{-1}. The alkyne signal induced from **2** was clearly mapped on the liposome membrane and well overlapped on the signal corresponding to C−C stretching vibration of lipid components at 1400 cm^{-1}.

The alkyne moiety of **2** facilitated Raman imaging for effective observation of cholesterol behavior in membrane. If the alkynyl group is generated under physiological condition, this probe would be more valuable. In looking for an alkyne formation reaction, we paid more attention to the reaction well-working in aqueous media as well as under a mild condition where membrane is not disturbed. We focused on Eschenmoser-Tanabe fragmentation, which was used at the second step in the preparation of **2**. An aliphatic ring containing an α,β-epoxy ketone moiety is opened by addition of *p*-toluenesulfonyl hydrazine ($TsNHNH_2$), resulting in production of an alkyne group. In our case, a cholesterol oxidized on the A-ring, 4,5-epoxycholestan-3-one (**1**), was converted into an ring-A-opening alkyne form **2** in aqueous media at room temperature.

The alkyne formation occurs efficiently even on a lipid bilayer membrane. We prepared liposomes including **1** and investigated the alkyne formation reaction on the liposomes and subsequent labelling with an azide-substituted fluorescein through copper-catalyzed click chemistry. As a result, only in the case with treatment of $TsNHNH_2$, the emission from the tethering fluorescein was clearly detected on the pellet of liposomes after centrifugation. The fluorescence was also microsopically observed on the lipid membrane of the liposomes. The precursor **1** efficiently reacted on the liposome membrane in the presence of $TsNHNH_2$ to convert into the alkyne form **2** and the liposome was labeled with azide-substituted fluorescein through copper-catalyzed Huisgen cyclization. The Raman signals of **2** formed by chemical conversion of **1** on membrane were also observed using the giant liposomes with the size of several micrometers. The lipid membrane constituents consisting of DOPC (40%), DPPC (40%) and **1** (20%) was suspended in a phosphate-buffered saline with a small amount of biotin-modified poly(ethylene glycol)-lipid (0.20%). The produced giant liposomes, which were immobilized onto the streptavidin-coated quartz substrate of the dishes through the biotin−streptavidin interaction, were observed with a Raman microscope before and after treatment of $TsNHNH_2$ for 40 min. After treatment of $TsNHNH_2$, the sharp signal appeared at the wavenumber of 2100 cm^{-1} in the Raman spectra of the whole image including the liposome, which were obtained by multicomponent analysis. On the other hand, before $TsNHNH_2$ treatment, there was no signal at the same wavenumber in the major components separated by the multicomponent system. The new peak at 2100 cm^{-1} was the signal contributed by the alkyne moiety of the product **2**.

In this study, a ring-A-opened alkyne steroid probe has been synthesized. This probe is Raman-active and postsynthetic-modification-acceptable on lipid bilayer membrane. In addition, chemical activation from the Raman-silent state of the ring-A-closed precursor is possible even on membrane. This multifunctional alkyne steroid probe would be a promising tool for understanding the intracellular distribution pathway and the environment-specific dynamics of cholesterol.

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🕮 IDENTIFICATION OF THE STRUCTURAL FEATURES OF NATURALLY OCCURING DAUCANE ESTERS EXHIBITING AN ANTI-INFLAMMATORY POTENTIAL

Višnja Popović[1,2,*], Jan Goeman[2], Jonathan Thommis[1], Nadia Bougarne[1], Arne Heyerick[3], Karolien De Bosscher[1,⊥], and Johan Van der Eycken[2,⊥]

[1] Receptor Research Laboratories, Nuclear Receptor Lab, VIB-Medical Biotechnology Center, Ghent University, Ghent, Belgium, [2] Laboratory for Organic and Bioorganic Synthesis, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium, [3] Reliable Cancer Therapies, Strombeek-Bever, Belgium
e-mail: visnja.popovic@ugent.be
[⊥]Shared senior authorship

This study is focused on naturally occurring sesquiterpene esters of the daucane type isolated from two *Laserpitium* L. (Apiaceae) species: roots and rhizomes of *L. latifolium* L. and the herb of *L. zernyi* Hayek. Some of the widely distributed species of the genus *Laserpitium*, such as *L. siler* L. and *L. latifolium* were traditionally used for the treatment of inflammatory and infectious diseases.[1,2] Despite the ethnopharmacological evidence, constituents of extracts of *Laserpitium* species were not tested for anti-inflammatory properties. Previous investigation showed that herbs of this genus were rich in daucane esters[3,4] as well as in guaianolide and eudesmanolide sesquiterpene lactones.[5]

Glucocorticoids (GCs), steroidal hormones that bind to the glucocorticoid receptor (GR), effectively treat various inflammatory disorders via inhibition of pro-inflammatory transcription factors nuclear factor (NF)-κB and activator protein (AP)-1. Unfortunately, their continuous use is associated with detrimental side effects such as e.g. the onset of diabetes.[6] Therefore, the search for novel molecules that can inhibit NF-κB and AP-1 activities and elicit anti-inflammatory effects without metabolic side effects is gaining more attention.

The aim of the present study was to test sixteen isolated daucane esters for their ability to inhibit pro-inflammatory NF-κB and AP-1 pathways. The most promising candidates were tested for their inhibition of concomitant inflammatory protein excretion and expression of specific genes induced upon a state of cellular inflammation.

In the current study, sixteen daucanes were isolated (flash chromatography and prep-HPLC) and their structure was elucidated (NMR, HR-MS and IR data). Apart from known compounds, hereby validating our approach, nine new molecules were isolated from the extracts of the two *Laserpitium* species. Two novel jaesckeanadiol derivatives (**1-2**) together with known compounds laserpitin (**5**) and siol-angelate (**10**) were isolated from the herb of *L. zernyi*. From the same extract, four novel daucanes were isolated – a compound featuring the keto-group at C-9 and Δ7,8 double bond (**11**), a daucane ester with a hydroxyl group at position C-7 (**12**), a molecule with a 8α,9α-epoxy-bridge (**15**) and a daucane featuring an exo-methylene group at C-8 (**16**). Two desoxodehydrolaserpitin analogues (**3-4**) and series of laserpitin (**5-8**) analogues were isolated from the underground parts of *L. latifolium*. Two more jaesckeanadiol esters (**13-14**) and a novel daucane featuring an α-oriented hydroxyl group (**9**) were isolated from the underground parts of *L. latifolium*. The chemical structures of compounds **2**, **3**, **4** and **9** are shown in **Figure 1.**

Anti-inflammatory activities were tested using a TNF-induced stably integrated recombinant NF-κB-dependent luciferase reporter gene construct, as well as a PMA-induced stably integrated recombinant AP-1-dependent luciferase reporter gene readout, both in A549 human lung endothelial cells. Such assays represent a powerful search tool for novel anti-inflammatory compounds. The compounds were screened at three concentrations (60, 30 and 10μM), which enabled to distinguish general from specific activity. Also, cell viability assays (Cell Titer Glo and NeoLuc A549 cells) were performed to distinguish cell toxicity from true gene repression. The most pronounced effects were exhibited by derivatives of 8-daucene-2,4,10-triol featuring an ester moiety at C-2 and C-10 (**2**, **3**, **4**) and by a compound featuring an additional α-

oriented hydroxy group at C-9 (**9**). Compounds without an ester at C-2 or C-10, as well as laserpitin analogues showed moderate activity, while a compound with an exo-methylene group at C-8 and a 8α,9α-epoxy-daucane exerted the weakest effects. Entities that emerged as most promising in reporter gene assays were further tested for their ability to inhibit pro-inflammatory CCL-2 chemokine production (as determined by ELISA) in the basal A549 cell line.

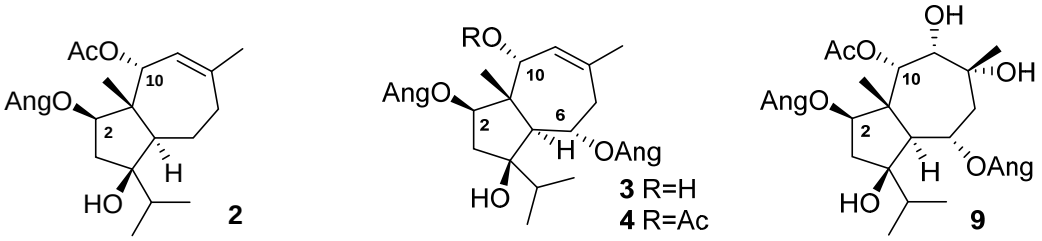

Figure 1. Structures of the compounds **2**, **3**, **4** and **9**.

Effects on specific gene expression were tested using qPCR and normalization to a series of housekeeping genes (via GENORM) in basal A549. The most active compounds showed a significant decrease in CCL-2 production, albeit to a lesser extent than the GC dexamethasone, a golden standard in the treatment of inflammation. Also, compound **9** produced a statistically significant inhibition of IL-6 and IL-1β gene expression, as determined by qPCR.

These results suggest which structural features in the daucane scaffold may be exhibiting anti-inflammatory activities, which could be further hints supporting a search for novel anti-inflammatory agents.

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🕮 PKA VALUES OF HYODEOXYCHOLIC AND CHOLIC ACIDS IN THE BINARY MIXED MICELLES SODIUM-HYODEOXYCHOLATE – TWEEN 40 AND SODIUM-CHOLATE – TWEEN 40: THERMODYNAMIC STABILITY OF THE MICELLE END THE COOPERATIVE HYDROGEN BOND FORMATION WITH THE STEROID SKELETON

Mihalj Poša*, Ana Pilipović, Mirjana Bećarević, Zita Farkaš, and Nataša Milić

Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

Due to a relatively small size of bile acid salts[1], their mixed micelles with nonionic surfactants are analysed. Of the special interest are real binary mixed micelles that are thermodynamically more stable than ideal mixed micelles. Thermodynamic stability is expressed with an excess Gibbs energy (G^E) or over an interaction parameter (β_{ij})[2]. In this paper sodium salts of cholic (C) and hiodeoxy-

cholic acid (HD) in their mixed micelles with Tween 40 (T40) are analysed by potenciometric titration[3] and their p*Ka* values are determined. Examined bile acids in mixed micelles with T40 have higher p*Ka* values than free bile acids. The increase of Δp*Ka* acid constant of micellary bound C and HD is in a correlation with absolute values of an interaction parameter. According to an interaction parameter and an excess Gibbs energy, mixed micelle HD – T40 are thermodynamically more stable than mixed micelles C - T40.

Δp*Ka* values are higher for mixed micelles with Tween 40 whose second building unit is HD, related to the building unit C. In both micellar systems, Δp*Ka* increases with the rise of a molar fraction of Tween 40 in binary mixtures of surfactants with sodium salts of bile acids.

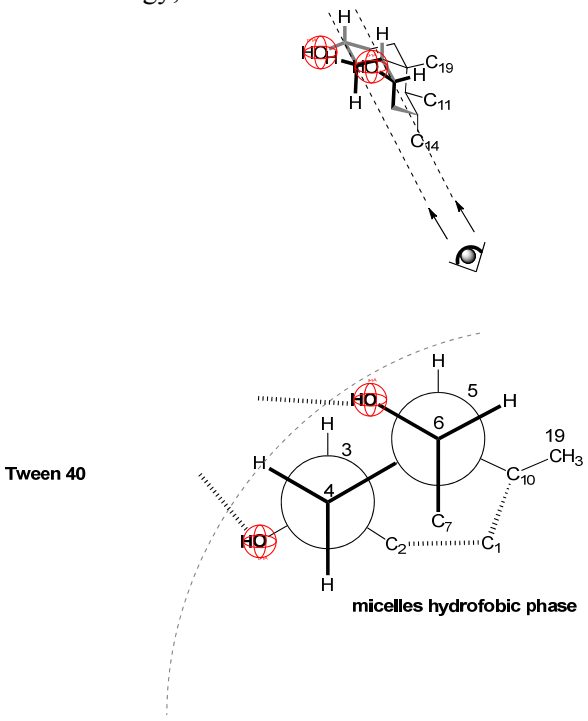

Fig 1. H-bonds beetwen Tween 40 and HD in their mixed micelles

This suggests that, Δp*Ka* can be a measure of a thermodynamic stabilisation of analysed binary mixed micelles as well as an interaction parameter. Δp*Ka* values are confirmed by determination of a distribution coefficient of HD and C in systems: water phase with Tween 40 in a micellar concentration and 1-octanol, with a change of a pH value of a water phase.

Conformational analyses suggests that synergistic interactions between building units of analysed binary micelles originates from formation of hydrogen bonds between steroid OH groups and polyoxiethylene groups of the T40. Relative similarity and spatial orientation of C_3 and C_6 OH group allows cooperative formation of hydrogen bonds between T40 and HD – an excess entropy in formation of mixed micelle. If a water solution of analysed binary mixtures of surfactants contains urea in concentration of 4M significant decreases of an interaction parameter value happens which confirms the importance of hydrogen bonds in synergistic interactions (urea compete in hydrogen bonds)

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🕮 24-EPIBRASSINOLIDE PHARMACOKINETIC STUDIES

Alina L. Sauchuk*, Raisa P. Litvinovskaya, Vladimir Nasek, and Elena Sanko-Schislenok

Institute of Bioorganic Chemistry NAS of Belarus, 220141 Minsk, Kuprevich Str.,2/5, Belarus
e-mail: alina_kyrtikova@mail.ru

Brassinosteroids (BS), which presently include over 80 compounds isolated from natural sources, are steroid plant hormones that play an important regulatory role in various physiological processes, such as growth, development, and reproduction of plants, adaptation to the environmental conditions. Therefore, BS are a promising new class of environmentally-friendly compounds for agriculture. Research on fundamental and applied aspects of BS is in rapid development. Results of these studies have already found practical applications in the design of the first representatives of a new generation of agricultural chemicals (Epin, Epin-Plus) based on the hormones of this group, which are currently used to increase plant productivity.

The finding of the protective and growth-activating effects of BS in fishes stimulated a systematic search of BS-initiated responses in other vertebrates and warm-blooded animals. The first results were obtained in the course of toxicological studies in rodents. They showed that BS influence the reproductive sphere, steroid hormonal balance, some biochemical and physiological parameters which reflected clear tendency to stimulative and adaptive shifts in experimental animals. Similarity of BS action in plants and in non-plant organisms displayed the potential value of these compounds for medicinal applications and initiated intensive studies.

Recent studies have shown stimulation,[1] antiviral,[2] neuroprotective,[3] antidiabetic action,[4] adaptogenic and other beneficial pharmacological effects of BS. There is also evidence that BS can be used in treating androgenassociated conditions, such as benign prostatic hyperplasia and androgenic alopecia.

Encouraging results were obtained in studies on the effects of BS on cholesterol level.[5] Application of 24-epibrassinolide (EBl) in daily doses of 2-200 μg/kg for 36 weeks to rats with normal blood cholesterol level gave 9-25% lower cholesterol depending on a dose in a manner, where higher doses corresponded to a higher cholesterol lowering effect. A similar trend towards decreasing cholesterol level on BS application was also observed in humans.[6]

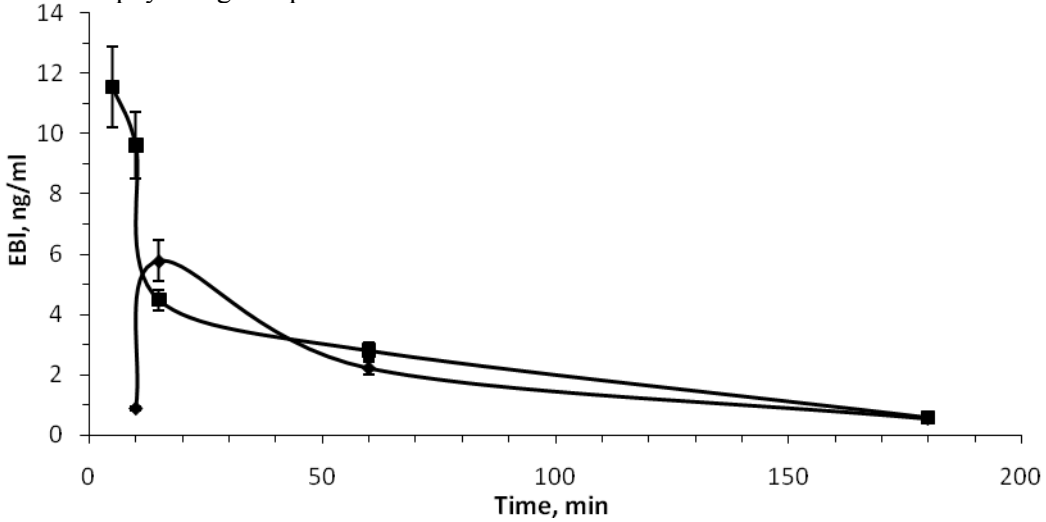

Fig. 1 - Plasma concentrations (mean ± SD) of EBl after intragastric **(♦)** and intraperitoneal **(■)** administrations at a dose of 0.75 μg/g

Table 1 - Results of pharmacokinetic study

Parameter	Intragastric administration	Intraperitoneal administration
Absorption rate constant (k_a, h^{-1})	11.2	–
Half-absorption period ($t_{1/2a}$, h)	0.062	–
Peak plasma concentration (C_{max}, ng/ml)	5.77	–
Time to reach C_{max} (T_{max}, h)	0.250	–
Expected initial concentration (C_0, ng/ml)	5.95	13.84
Volume of distribution (V_d, l/kg)	–	72.2
Apparent volume of distribution (V_d, l/kg)	168.1	–
Area under curve C(t) in plasma (AUC, ng·h/ml)	5.19	14,7
Elimination rate constant (k_e, h^{-1})	0.836	0.943
Terminal half-life ($t_{1/2e}$, h)	0.829	0.735
Total clearance (Cl, l/h/kg)	–	68.0
Mean residual time, (MRT, h)	1.29	1.06
Mean absorption time (MAT, h)	0.23	
Bioavailability (F, %)	35.3	

Therefore, in view of BS importance in medical practice the aim of this research was to study their pharmacokinetics. Plasma concentration profiles and pharmacokinetic parameters have been obtained following single dose intragastric or intraperitoneal administration of EBl (150 μg per 200 g) in Wistar rats.

A direct ELISA for plasma EBl was developed. The EBl calibrating samples (0-100 nM) were prepared in intact plasma by dilution from a stock solution of 10^{-4} mol/l in ethanol. Labeled antigen solution was prepared by dilution in assay buffer. The calibrating samples (0-100 nM) or the plasma samples (50 μl in duplicates) and the enzyme conjugate (100 μl per well) were added to the wells of the plate with the immobilized antibodies. Further procedures were carried out as in the traditional ELISA.[7]

Pharmacokinetics of EBl was characterized by areas under EBl plasma concentration-time curve (Fig. 1) after intragastric and intraperitoneal administrations. EBl was well absorbed from the gastrointestinal tract following the administration and quickly distributed to blood, liver, intestines, lungs and kidneys. The blood plasma highest concentration was reached in 5-10 min after administration. The plasma elimination half-life of EBl was about 50 min after administration. Results of pharmacokinetic study are shown in Table 1

Concentration of EBl in various organs was also determined. The highest concentration in liver took place after 60 min and then it gradually decreased. The quickest EBl-accumulating organ was found to be small intestine, where only 20 min were needed to reach its highest concentration. Other organs (kidneys, spleen, lungs and heart) accumulated EBl to a little degree. The elimination of EBl has been studied. The obtained data will be discussed.

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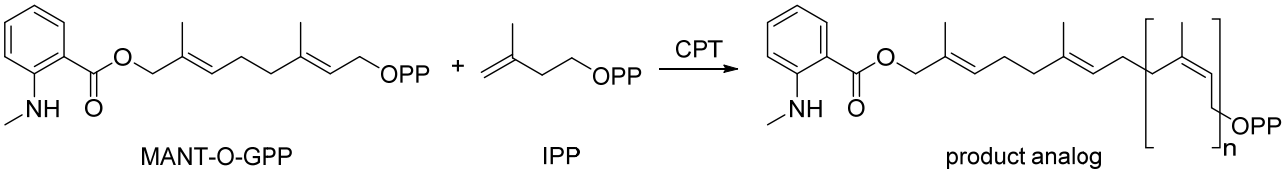
🕮 ENZYMATIC SYNTHESIS OF ARTIFICIAL POLYPRENOLS

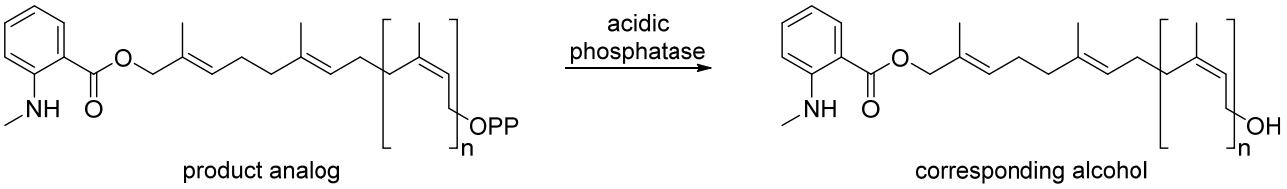
Pia Schöne, Jeanette Keim, Steve Ludwig, and Ludger A. Wessjohann*

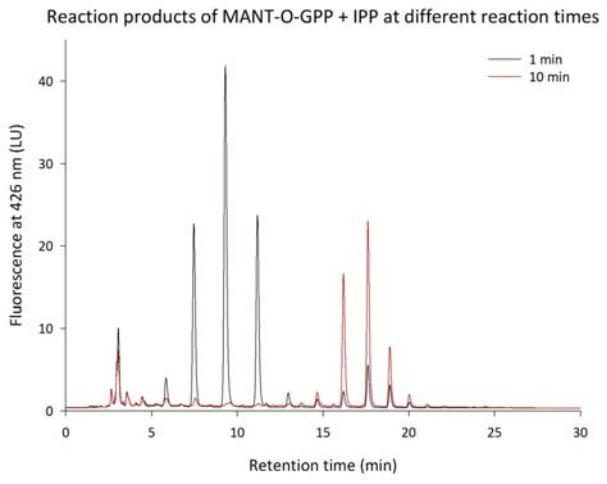
Leibniz-Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Weinberg 3, D-06120 Halle, Germany
e-mail: wessjohann@ipb-halle.de

Magnesium-dependent cis-prenyltransferases (CPT) catalyze the consecutive addition of homoallylic isopentenyl diphosphate (IPP) monomers onto allylic farnesyl diphosphate (FPP) to form *cis*-configured linear polyprenyl diphosphates[1]. These native products, as well as derivatives of them, are interesting for industrial processes like the production of neoprene (a long-chained chloroprene).

Medium-chain CPTs are able to elongate FPP by 8-10 molecules of IPP[2,3] and the chain elongation can be monitored using the fluorescent allylic starter substrate MANT-O-GPP[4]. Some CPTs are also known to accept artificial substrates like halogenated homoallylic diphosphates but until now, a condensation of more than two of such substrates has not been reported, since halogens in the activated prenyl unit were proven to inhibit terpene synthases[5].







We identified a thermophilic CPT from *Thermococcus kodakaraensis* (ThkCPT) that is able to catalyze the addition of IPP as well as an unprecedented oligomerization with 3-chlorobut-3-enyl diphosphate (Cl-BPP) monomers onto MANT-O-GPP.

The corresponding product alcohols could be detected using a fluorescence based HPLC method and the chain length of the resulting oligochloroprenes was proved by high resolution mass spectrometry. The distribution analysis showed up to 12 condensations of IPP and up to 8 condensations of Cl-BPP. Interestingly, the choice of the bivalent cation as well as the use of dual-phase systems changes the distribution of differently sized products significantly. Thus, the accumulation of specific products can be achieved by controlled modulation of the reaction conditions.[6]

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🕮 UPTAKE AND METABOLISM OF FLUORESCENT CHOLESTEROL ANALOGUES BY MYCOBACTERIAL CELLS

Yaroslav Faletrov[1,2], Anna Brzostek[3], Renata Plocinska[3], Jarosław Dziadek[3], Elena Rudaya[1,2], Irina Edimecheva[1], and Vladimir Shkumatov[1,2]*

[1] Research Institute for Physical Chemical Problems, Belarusian State University, Minsk, Belarus; [2] Faculty of Chemistry, Belarusian State University, Minsk, Belarus; [3] Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland.
e-mail: vlad.shkumatov@tut.by

Mycobacteria are known to take up and metabolize cholesterol and for *Mycobacterium tuberculosis* (Mtb), a severe pathogen, the processes are thought to be important for survival and virulence. Thus, modified cholesterol-like molecules might be useful as novel anti-tubercular agents or probes for monitoring of the axis of host-pathogen interaction. On the other hand, common fluorescent cholesterol analogues, 22-NBD-cholesterol (22NC), 25-NBD-cholesterol (25NC) and TopFluor™ BODIPY-cholesterol (BPCh), are known to be powerful probes for studying various biological processes. Previously we found that 22NC and 25NC (**Fig. 1**) are substrates for a cholesterol dehydrogenase from an actinobacterium Nocardia sp.[1] and such enzymes are known to start cholesterol degradation by mycobacteria.[2]

For better understanding of such steroids interaction with we synthesized and used in our test two new NBD-labeled cholesterol-like molecules, namely 20NP (a 22NC homologue, HPLC-separated 20*R*-isomer) and 3NC (a cholestane with NBD fragment instead of 3-OH group; HPLC separated 3α-H) (**Fig. 1**). The compounds from pregnenolone and 5α-cholestan-3-one using reductive amination approach (NH_4OAc + either $NaBH(OAc)_3$ or $NaBH_3CN$),[3] followed by HPLC separation of pure isomers. The compounds were obtained with affordable yield (12 % and 15 %, respectively) and their structures were confirmed by various methods.

We test BPCh, 22NC and newly synthesized 20NP and 3NC interactions (uptake and metabolism) with wild type Mtb $H_{37}Rv$ and *M. smegmatis* (Msmeg) mc^2, cultivated on a minimal medium with glycerol as a single carbon source.

It has been found that BPCh, 22NC and 20NP undergo bioconversions by the Mtb and Msmeg strains. The major fluorescent products were determined to be 4-en-3-one (P1) and, at less extent, 5-en-3-one (P2) derivatives of the analogues according to mass-spectrometry data, showing 2 Da loss in the products structures (m/z values for $[M-H]^-$ ions 573, 491 and 477 for the products, derived from BPCh, 22NC and 20NP) as well as comparison of major products of BPCh and 20NP conversions by pure cholesterol dehydrogenase from *Nocardia sp.* (a homologue of the mycobacterial 3β-HSDs) (**Fig. 2**).

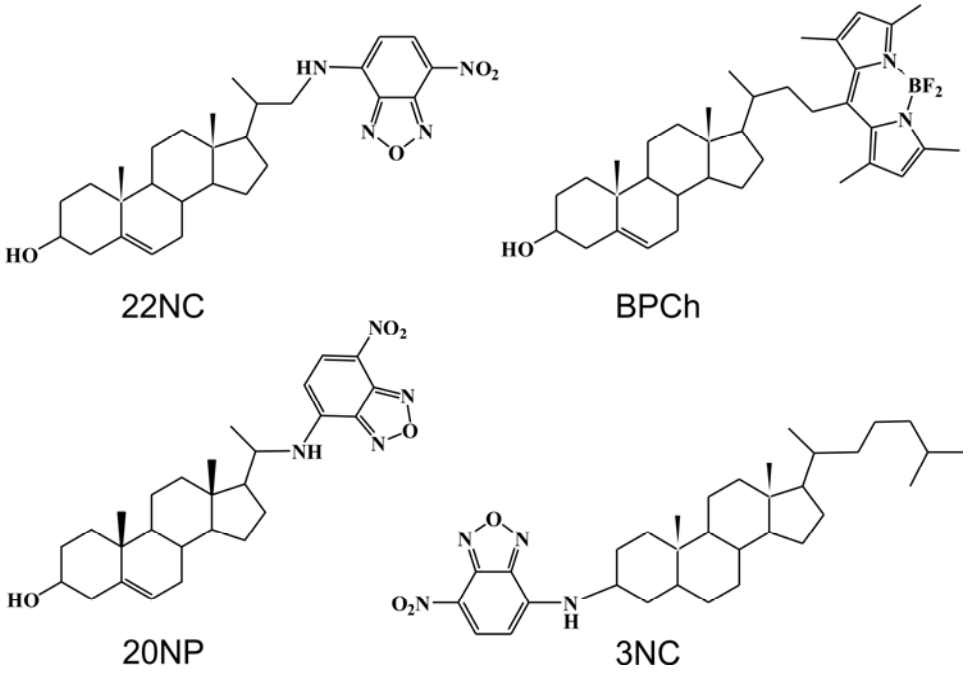

Fig. 1. Structures of the fluorescent steroids tested.

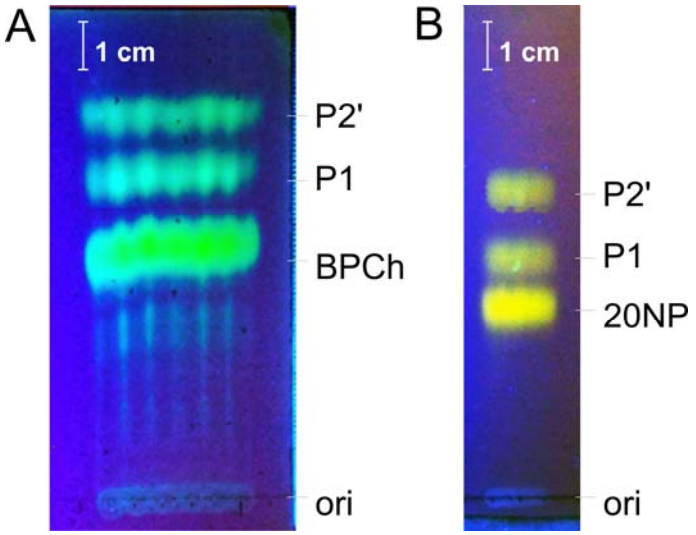

Fig. 2. A photo of TLC plate, showing separation of the P1-type and P2'-type products formed during incubation of BPCh (A) and 20NP (B) (25 μM both) with NAD^+ (1 mM) and cholesterol dehydrogenase (3β-HSD) from *Nocardia sp*. (0.35 U/ml). P1 and P2' designate the 4-en-3-one and 5-ene-3-one derivatives of the fluorescent substrate, respectively.

Destruction of NBD fluorophore of 22NC and 20NP was also detected. Neither NBD destruction nor steroidal part modification were observed for the synthesized 3-(NBD)-cholestane, which is in accordance with inability to be converted by the cholesterol dehydrogenase and inertness of a similar cholestane in mycobacteria.[4]

According the fluorescent microscopy data the compounds were found to stain both membrane lipids and cytosolic lipid droplets (LDs) of the my-

cobacteria, and the ability was pronounced for the most lipophilic 3NC and BPCh among the compounds' set (Hyperchem 8.0 computed Log P values 5.34, 5.65, 9.08 and 8.19 for the structures of 20NP, 22NC, 3NC and BPCh, respectively).

Recent publications describing association of cholesterol with a fluorophore-substituted 3-OH-group with mycobacterial lipids[4] and metabolism/growth inhibition of side-chain modified (fluorinated) cholesterols[5] by Mtb have demonstrated actuality of the research performed, emphasizing further research efforts in the studies of modified steroids interactions with mycobacteria.

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🕮 ANTIHYPERLIPIDEMIC EFFECT OF *MORUS ALBA* L. ON STREPTOZOTOCIN-INDUCED DIABETIC ADULT MALE WISTAR RATS

Jalal Solati and Elaheh Gholami Roudmajani

Department of Biology, Karaj branch, Islamic Azad University, Karaj, Iran
e-mail: jsolati@gmail.com

White Mulberry (*Morus Alba* L.) has had all parts of it used in traditional medicine for a variety of purposes, but currently most evidence on Morus Alba is in regards to its anti-diabetic properties.[1,2] We have investigated the effects of lipophilic (isoprenoids) extracts from Morus Alba *L.* fruit on serum triglycerides, LDL-cholesterol, HDL-cholesterol and activities of aminotransferase enzymes in streptozotocin-induced diabetic adult male rats. Continuous supplementation of lipophilic extract by gavage at doses of 0.25, 0.25 and 1 g/kg in 0.5 ml distilled water in diabetic rats resulted in a significant decrease of LDL-cholesterol and triglyceride levels after 14 days. The levels of HDL-cholesterol and activities of serum aminotransaminase enzymes, alanine aminotransferase and aspartate aminotransferase were not changed significantly in the extract-supplemented group compared to the control group.

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🕮 DEVELOPMENT OF MYCOBACTERIAL STRAINS PRODUCING TESTOSTERONE

Nicolai Strizhov*, Mikhail Karpov, Galina Sukhodolskaya, Vera Nikolayeva, Victoria Fokina, Andrei Shutov, and Marina Donova

G. K. Skryabin Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, 142290, Russia
e-mail: strizhov@ibpm.pushchino.ru

Testosterone (androst-4-en-17β-ol-3-one) is the main male sex hormone, an anabolic steroid as well as a drug and precursor of other important steroid drugs. Conventionally, it is produced by chemical synthesis. Whole-cell microbial biocatalysis application for testosterone production by side-chain degradation of sterols (e.g. cheap and available phytosterol, or cholesterol) is still in initial stage of development. Previously, utilization of androsta-1,4-diene-3,17-dione (ADD) - producing *Mycobacterium* sp. VKM Ac-1816D allowed us to obtain testosterone in a single biotechnological operation from β-sitosterol.[1] However, the rate of bioconversion (β-sitosterol to testosterone) did not exceed 50% level.

For further improvements heterologous 17β-hydroxysteroid dehydrogenases/reductases (17β-HSDs) catalyzing the key reaction of androst-4-ene-3,17-dione (AD) reduction to testosterone (Fig. 1) were introduced into mycobacterial cells. DNA sequences encoding three different 17β-HSDs (fungus *Cochliobolus lunatus*, human - 17β-HSD type 3, mouse - 17β-HSD type 5) were designed *in silico*.

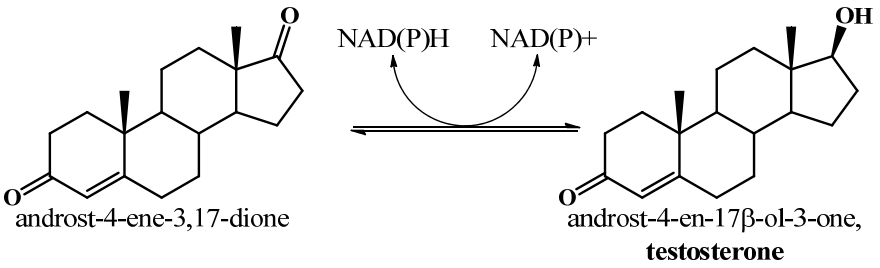

Fig. 1. 17β-Hydrogenation (reduction) of androst-4-ene-3,17-dione (AD) to testosterone by 17β-hydroxysteroid dehydrogenases/ reductases (17β-HSDs). NAD(P)H is used as a coenzyme.

Original cDNA and protein sequences were taken from the Database GenBank NCBI: ACCESSION AF069518, *Cochliobolus lunatus* 17β-hydroxysteroid dehydrogenase (17β-HSDCl); ACCESSION NP_000188, *Homo sapiens* testosterone 17β-dehydrogenase (17β-HSDHs); ACCESSION P70694, *Mus musculus* Estradiol 17β-dehydrogenase 5 (17β-HSDMm).

All three original cDNA sequences (fungus, human and mouse) have been re-encoded and optimized (spectrum of codon usage, RNA secondary structures; all codons rarely used and intragenic Shine-Dalgarno sequences have been removed) for high expression in mycobacteria. GC content was increased to 62.6% (fungus), 58.1% (human), 59.0% (mouse) compared to 57.4%, 47.5%, 43.8%, respectively, in the native sequences.

In order to shift the redox potential in cells towards reduction for conduction of hydrogenation reactions (in our case the hydrogenation of AD to testosterone) the genes encoding glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) were also introduced in mycobacteria for controlled expression of these dehydrogenases supplying a hydrogen donor NAD(P)H. *Mycobacterium tuberculosis* H37Rv served as a source of the genes for G6PD (Rv1121, zwf1; Rv1447c, zwf2) and for 6PGD (Rv1122, gnd2).

Prior expression and functional analysis carried out in *Escherichia coli* bacteria demonstrated that the constructed recombinant plasmids (on basis of pET28 vector) provided a high expression level of heterologous proteins and heterologous 17β-oxosteroid reductases produced are capable of reducing AD to testosterone *in vitro*.

Two sets of mycobacterial constructs were made: bicistronic with two genes combined (17β-HSD and G6PD) and monocistronic, where the genes were placed under control of the inducible acetamidase promoter. Recombinant *Mycobacterium smegmatis* mc^2155 strains expressing heterologous 17β-HSDs (fungal 17β-HSDCl, human17β-HSDHs, murine 17β-HSDMm) have been constructed. Generated mycobacteria reduced AD to testosterone. The highest testosterone production level achieved is 0.53 g/l at 1.0 g/l AD load (53% conversion) in case of fungal 17β-HSDCl coexpressed with glucose-6-phosphate dehydrogenase G6PDMt2 from *M. tuberculosis*.

Testosterone and boldenone (androsta-1,4-dien-17β-ol-3-one) (Fig. 2) were produced by recombinant mycobacteria when ADD was applied as a substrate for bioconversion. Complete ADD conversion to these products was reached with use of bicistronic construct encoding 17β-HSDCl and G6PDMt2.

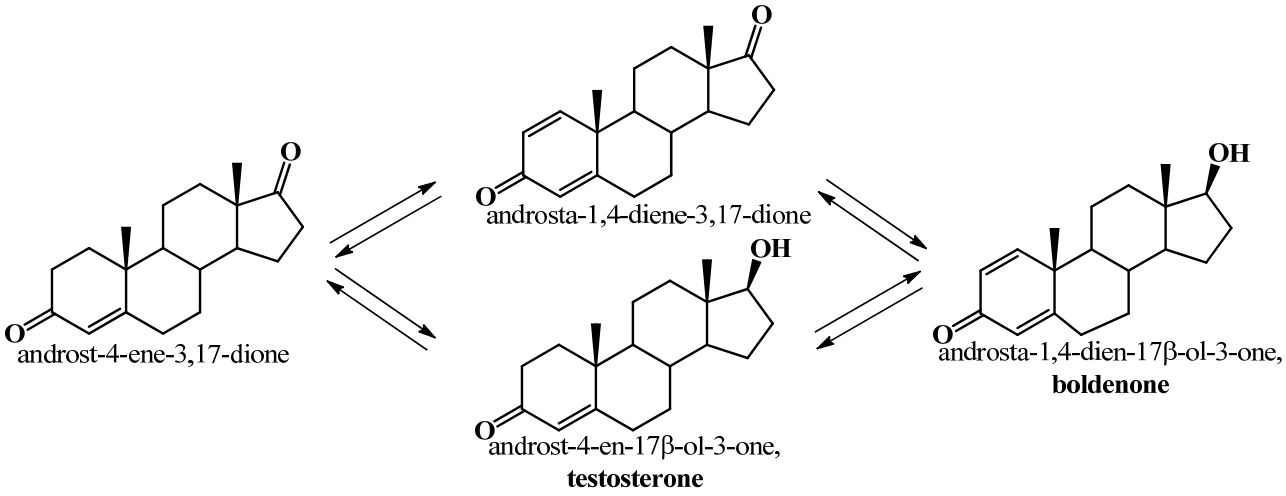

Fig. 2. Interconversions of AD (androst-4-ene-3,17-dione), ADD (androsta-1,4-diene-3,17-dione), boldenone (androsta-1,4-dien-17β-ol-3-one) and testosterone (androst-4-en-17β-ol-3-one) by 17β- and 1- oxidation/reduction.

Substantial quantities of testosterone can be produced by *M. smegmatis* mc^2155 from both substrates AD and ADD using the own innate reductive capacities for 17β- and 1-hydrogenation (Fig. 2) i.e. without heterologous 17β-HSD, on condition of supplying required amount of reduced NAD(P)H (constructs with G6PDMt1, 2 and 6PGDMt only).

Recombinant *Mycobacterium neoaurum* VKM Ac-1816D strain (normally used as ADD producer) with plasmid pNS25 encoding fungal 17β-HSDCl and G6PDMt2 efficiently converted β-sitosterol to testosterone and essentially outperformed wild type non-recombinant VKM Ac-1816D in terms of yield as well as completeness of bioconversion and by-product amount.

The recombinant strains constructed can be used as a platform for effective single-step biotechnological testosterone production process from phytosterol.

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🕮 STRUCTURE-FUNCTION ANALYSIS OF HUMAN MITOCHONDRIAL P450 STEROID HYDROXYLASES

Natallia V. Strushkevich[1*], Irina P. Grabovec[1], Limin Shen[2], Farrell MacKenzie[2], Tatiana Cherkesova[1], Andrei A. Gilep[1], Sergey A. Usanov[1], and Hee-Won Park[3]

[1] Institute of Bioorganic Chemistry NAS of Belarus, Kuprevich Street, 5/2 Minsk, 220141 Belarus; [2] Structural Genomics Consortium, University of Toronto, 101 College Street, Toronto, Ontario, M5G 1L7, Canada; [3] Tulane University School of Medicine, Department of Biochemistry and Molecular Biology, New Orleans, LA 70112, USA.
e-mail: natstrush@gmail.com

Steroid hormone biosynthesis is the essential pathway in eukaryotes. The mitochondrial CYP-dependent monooxygenase system represents a unique electron transport chain absolutely required for the production of steroids hormones as well as vitamin D derivatives and bile acids. Disturbances in this system due to congenital defects in the enzymes or high sensitivity to xenobiotics can cause endocrine disorders. Here we provide structural insights into the first and last steps of corticosteroid hormones synthesis. A series of structures of members of the CYP11 family reveals how active site can accommodate C27 and C21 steroids without major amino acid substitutions. However, mechanism of multistep catalysis might be different between CYP11A1 and CYP11B but similar among other mitochondrial CYPs. Notably, the active site pocket is lined by identical residues between CYP11B isoforms implying substantial conformational flexibility imparted by amino acid substitutions outside the active site. Considering that both CYP11B isoforms are drug targets it is challenging to develop a selective inhibitor. Studies of the CYP11B allostery are under way. Our structural complex of P450-ferredoxin provides important information about the intermolecular interactions within mammalian mitochondrial clan.

🕮 SOME RESULTS OF STUDY OF ECDYSTEROID-CONTAINING PLANTS OF KAZAKHSTAN

Borash I. Tuleuov

International research and production holding "Phytochemistry", Karaganda, Republic of Kazakhstan.
e-mail: phyto_pio@mail.ru

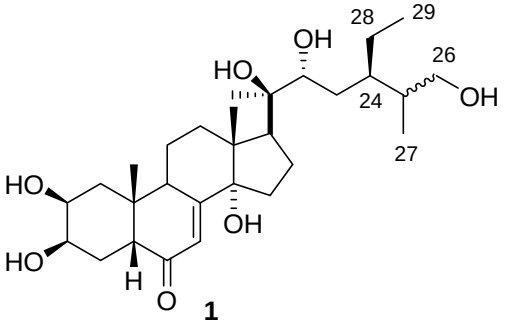
Ecdysteroids (ecdysones) – polyhydroxylated steroids, structural analogs of ecdysterone (20E) – are of interest as perspective sources of pharmaceuticals, first of all, of anabolic and adaptogenic action. The main advantage of the phytopreparations containing ecdysteroids as anabolic agents is their high pharmacological activity, lack of side effects: ecdysteroids, in particular ecdysterone, 25S-inocosterone, turkesterone and some other compounds do not show androgenic and gonadotrophic effects.

In this regard during the last decade IRPH "Phytochemistry" has been carrying out a wide screening of the Kazakhstan flora for the purpose of identification of superproducing species, biotesting and molecular model operation of activity of ecdysteroids for identification of the most important compositions.

In the phytochemical study of perspective representatives apart from ecdysterone and the known ecdysteroids we isolated a number of new and rare phytoecdysteroids, thus, from *Rhaponticum serratuloides* (Georgi) Bobr. we extracted a new S-29 ecdysteroid, containing an allylic hydroxyl group with the carbon atom S-29-rasersterone. (2β, 3β, 14α, 20R, 22R, 29-hexahydroxy-5β(H)-stigmast-7; 24(28)-diene-6-one) (**1)** and new ecdysteroid 25-epi-amarasterone A (2β, 3β, 14α, 20R, 22R, 29-hexahydroxy-5β(H)-stigmast-7-en-6-one) (**2)**, from the close species of the same genus – *Rhaponticum karatavicum* Regel et Schmalh., an endemic of the Republic of Kazakhstan, a stigmastan ecdysteroid having in its structure methylreplaced cyclic δ-

lactone fragment, consisting of five atoms of carbon and one atom of oxygen in position 24 and identified as reptansterone (**3)** was derived.

In the detailed study of aboveground portion of the plant *Acanthophyllum gypsophyloides* Regel. (bloodline *Caryophyllaceae Juss.*) were found 20-hydroxyecdysone and a new holestane phytoecdysteroid 3α, 14α, 22R, 25-tetrahydroxy-5α(H)-holest-7-en-6-one (**4)**, which obtained a trivial name acantosterone.[1]



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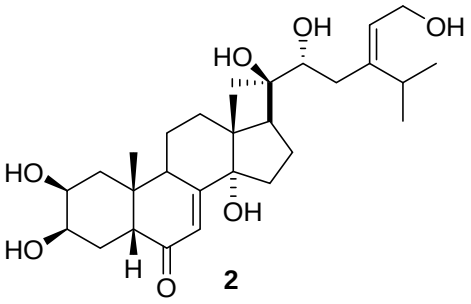

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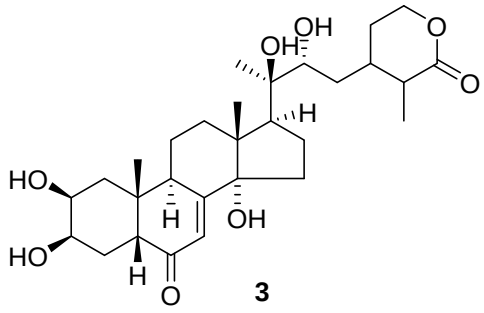

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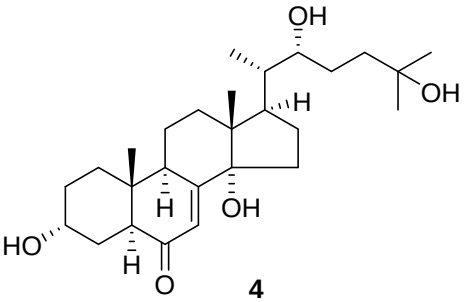

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🕮 ACCESS TO FUNCTIONALIZED EXTRANUCLEAR HETEROSTEROIDS *VIA* MODIFIED ACID THIOHYDRAZIDES

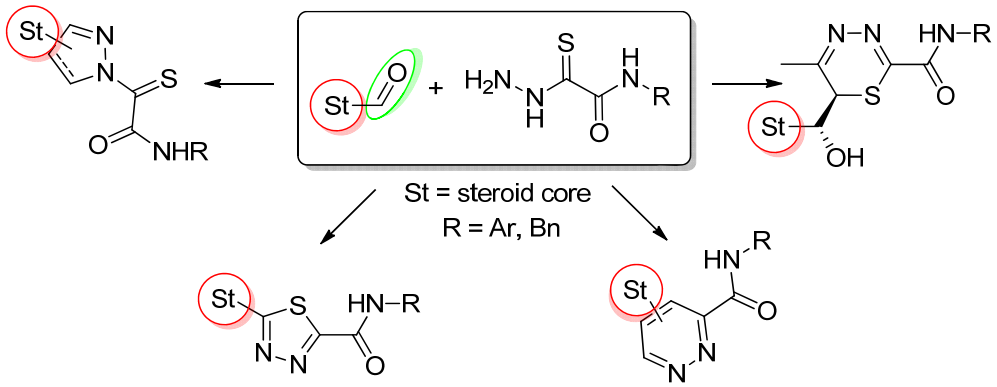
Yulia A. Volkova,*[1] Yaroslav S. Antonov,[1] Alexander V. Komkov,[1] Alexander M. Scherbakov,[2] Elena I. Chernoburova[1], and Igor V. Zavarzin[1]

[1] N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russia, [2] N.N. Blokhin Cancer Research Centre, Ministry of Health of the Russian Federation, 24 Kashirskoe sh., 115478 Moscow, Russia
e-mail: yavolkova@gmail.com

Extranuclear heterosteroids, over the years, have been considered to be privileged scaffold for drug discovery due to their outstanding biological activity, which is especially true for A- and D-ring modified steroids.[1] Thus, synthetic azasteroids encompass a wide range of compounds with various biological activities, e.g., reductase inhibitors such as finasteride, the high-affinity agonist ligands for the glucocorticoid receptor e.g., cortivazol, GnRH agonists such as danazol, aromatase inhibitors such as formestane and exemestane and neuromuscular junction blocking agents such as pipecuronium.

This study was focused on search of a facile flexible strategy, in which a common interme-

diates could be used in a conjunctive fashion to form an array of structurally diverse N-heterocycles attached or fused to a steroid core. In this regard we studied oxamic acid thiohydrazides as simple "versatile agents" for the modification of steroids bearing a carbonyl group. Namely, a flexible approach to unknown pyrazole, 4,5-dihydro-pyrazole, 1,3,4-thiadiazole, thiadiazine, and pyridazine derivatives of steroids with selective control of heterocyclization patterns was disclosed.[2]



(N-arylcarbamoyl)spiroandrosten-17,6′[1,3,4] thiadiazines, (N-arylcarbamoyl)androsteno- and (N-arylcarbamoyl)-$\Delta^{1,3,5(10)}$-estra-trieno[16,17-d]pyridazines and etc., new types of heterosteroids, were prepared from 16,17-epoxy- and 17-chloro-16-formyl steroids in good/high yields by the treatment with oxamic acid thiohydrazides. The antiproliferative activity of some synthesized compounds was evaluated against the human estrogen-responsive breast cancer cell line MCF-7, as well as against the estrogen-independent breast cancer cell line MDA-MB-231, using the MTT assay.

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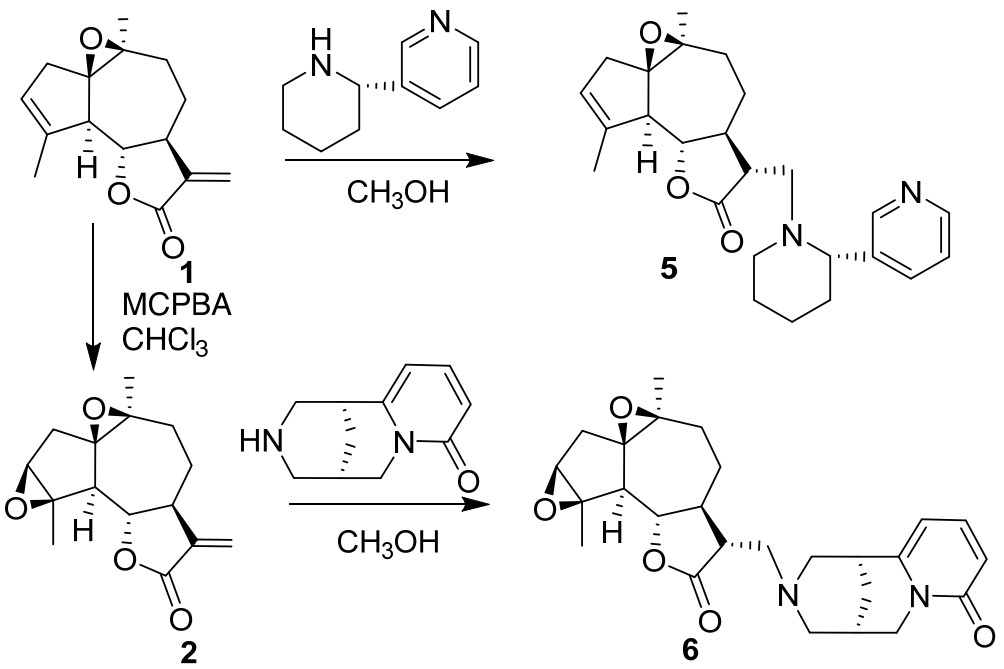
🕮 POSTERS

🕮 NEW DERIVATIVES OF ARGLABIN

Sergazy M. Adekenov, Anar S. Kishkentayeva, Baglan A. Abdygalymova, Dias N. Kokkozov, and Gayane A. Atazhanova

International Research and Production Holding "Phytochemistry", Karaganda Republic of Kazakhstan
e-mail: arglabin@phyto.kz

The sesquiterpene lactones containing atom of nitrogen show biological activities (anticancer, antibacterial). In plan of receiving nitrogen-containing derivatives we have carried out reactions of amination on the basis of a sesquiterpene lactone of an arglabin **1** and its derivative β-epoxyarglabin **2**. We used alkaloids anabasine **3** and cytisine **4** as amine, which possess the expressed pharmacological activity. As a result on the base of an arglabin new bimolecular derivatives **5, 6** with quantitative exits are received.



The structure of the received compounds **5, 6** is determined on the base of physical and chemical constants and spectral data (IR-, UV-, NMR ^{1}H and ^{13}C, two-dimensional ranges of a NMR ^{1}H-^{1}H (COSY, NOESY) and ^{13}C-^{1}H (COSY,COLOC).

🕮 NEW ANTI-STRESS ACTIVITY TEST FOR BRASSINOSTEROIDS

Vladimir I. Anikeyev and Alina L. Sauchuk*

Institute of Bioorganic Chemistry NAS of Belarus, 220141 Minsk, Kuprevich Str., 2/5, Belarus
e-mail: alina_kyrtikova@mail.ru

Brassinosteroids (BS) are endogenous plant growth-promoting hormones, which stimulate various physiological processes in plant organs including elongation, vascular differentiations, male fertility, timing of senescence, decelerates of leaf senescence and improves plant resistance to stresses [1]. Salinity is a major abiotic stress affecting growth, development and productivity of plants. It is known that BS exhibit a pronounced protective effect on plants under salt stress [2].

Our experiment was carried out using plants of winter wheat (cv. Caraway) and BS as anti-stress drugs.

Seeds were soaked in water solution (10^{-8} M) of 24-epibrassinolide (EBl) or brassinolide (Bl), or mixture (1:1) of EBl and Bl for one day. Control seeds were soaked in distilled water. Then the seeds were transferred on wetted with experimental solutions filter paper, which was rolled into rolls (3 rolls for each experiment). These rolls were put in a glass with water and kept in darkness (20°C). After 3 days rolls were taken out and deployed. Germination energy of wheat seeds was measured to be 83% (EBl), 90% (Bl), 83% (EBl + Bl) and 83% (control). Then non-germinated seeds were removed and the paper rolls were continued to be kept under the same conditions. After 5 days the rolls with wheat seedlings were exposed to light. They grew at room temperature with natural lighting for 6 days. The length of seedlings was measured every day. Then the rolls with seedlings were put in a glass with concentrated (1 M) solution of sodium chloride (NaCl). After 8 days the rolls were taken out and deployed, plants were washed with water (2 liters per roll) without removing them from the substrate. The rolls were placed in a glass with water and kept at room temperature.

The experimental results showed that the growth rate of the experimental and control plants are about the same. However plant survival value at 22 days was 56% for series EBl, 42% for series Bl, 36% for series EBl+Bl and 14% for the control sample. Moreover, 32% of the plants of the EBl series, 27% of the plants of the Bl series, 28% of the plants of the EB+Bl series, 0% of the control plants not only survived but continued natural growth and development giving the second leaf.

These results are the basis for the development of a relatively simple and cost-effective test for examination a number of new compounds of BS- series on the anti-stress activity.

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🕮 ISOPRENOID COMPOUNDS (POLYPRENOLS, MONO- AND SESQUITERPENOIDS OF ESSENTIAL OILS) OF PLANTS OF KAZAKHSTAN'S FLORA

Gayane A. Atazhanova

International Research and Production Holding "Phytochemistry", Karaganda, Republic of Kazakhstan
e-mail: arglabin@phyto.kz

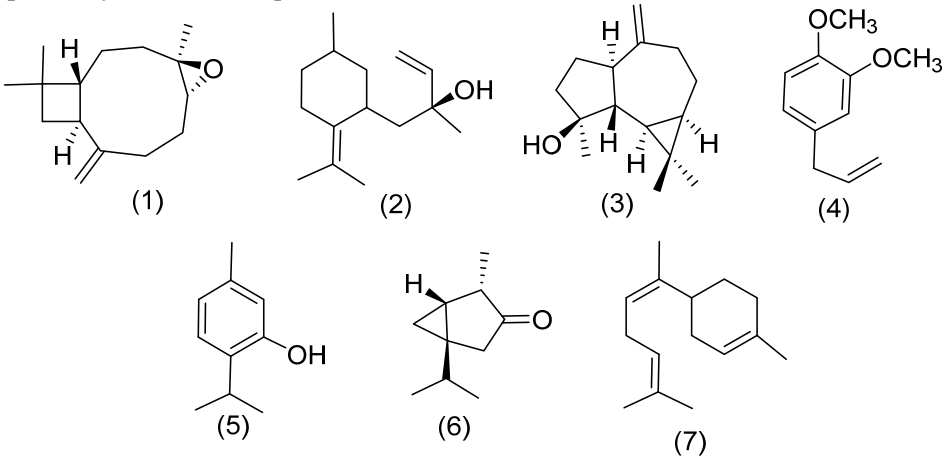
At the present time we have started the study of plants of Kazakhstan's flora on the content of the polyprenol compounds. So, chemical screening of extractive substances of 9 species of plants as *Pinus sylvestris* L., *Spiraeanthus schrenkianus* Maxim., *Spiraea hypericifolia* L., *Hippophae*

rhamnoides L.*, Lonicera tatarica* L.*, Hipericum perforatum* L.*, Betula pendula* Roth., *Fraxinus sogdiana* Bunge. and *Juniperus sabina* L. Revealed for the first time the content of the polyprenol compounds in *Spiraeanthus schrenkianus* Maxim; *Spiraea hypericifolia* L. and *Juniperus sabina* L. Bioscreening of 5 samples on biological activity is carried out; at the same time carbon dioxide extracts of *Pinus sylvestris* L., *Hippophae rhamnoides* L., bark *Popula tremula* and leaves of *Betula* have shown moderate hepatoprotector activity. We have developed the laboratory regulations and technology of production of polyprenol from 15-21 isoprene links from *Juniperus sabina* L.

Among secondary metabolites of plants the essential oils rich in contentof biologically active isoprenoids are of considerable interest. However, insufficiency of a source of raw materials limits the possibilities of their practical application that gives undoubted relevance to search of new plant sources to obtain them. By the GC-MS method we have established a chemical composition of essential oils from 46 species of plants. Essential oils isolated by hydrodistillation method with use of Clevenger's device, microwave extraction on «Millestone» coater and distillation with water vapor on trial installation.

The quantitative content of essential oils' components and their qualitative composition differed depending on the species of plants and used parts of plants. For further study we selected essential oils: Bupleurum longiforium Fisch. ex. Hoffm. and *Bupleurum multinerve* DC for producing caryophyllene oxide (1) (30.15%) and (60.9%), respectively, for subsequent isolation nerolidol (2) (26.2%) from the essential oil of *Scutellaria subcaespitosa* Pavlov, spathulenol (3) (57%) of the essential oil from Schrenkia congesta Korov, methyleugenol (4) (60.4%) of the oil from Dracocephalum karataviense Pavl., thymol (5) (69.7%) and (37.4%) of essential oils from Origanum tyttanthum L. and Thymus karataviense Dmitrieva ex Gamajun (37.64), β-thujone (6) (90%) of the essential oil from wormwood Artemisia abrotanum L., α-bisabolene (7) (40.42%) from Artemisia arenaria DC and others.

The result of screening for antimicrobial and cytotoxic activity found that the samples of essential oils of *Artemisia saissanica* Krasch. *, Artemisia monogyna* Waldst. & Kit.*, Artemisia ferganensis* Krasch. ex Poljakov exhibit moderately strong antibacterial activity against gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis*, and have cytotoxic activity. For the first time it established that the essential oils of *Thymus mugodzharicus* Klokov & Desjat.-Shost and *Thymus petraeus* Serg, exhibit strong antioxidant activity. In studying the ability to inhibit the infectious activity of the virus A/FPV/Rostock/34 (H7N1) was found that essential oils of *Thymus rasitatus* Klokov and *Thymus petraeus* Serg exceed the activity of commercial agents amizon and heviran more than 1,0 lg. The experiments established that the essential oils of *Thymus rasitatus* Klokov, *Ferula ceratophylla* Regel*, Artemisia proceraeformis* Krasch.*, Paeonia hybrida* Pall. and samples of hexachlorine derivative ocimene, tetrahlorcarbene derivative terpinene, 4-chloro-4-isopropyl-1- (2,2,2-trichloroethyl)-cyclohex-1-ene have a pronounced analgesic effect.



Researchers conducted on specific activity concerning *Mycobacteria tuberculosis* of a strain H37RV of essential oil from *Artemisia glabella* Kar. et Kir. and a preparation on its basis («Epherol» spray) on model of chronic pulmonary tuberculosis of experimental animals.

Thus, the analysis of the researches conducted by us characterizes plant terpenoid a (polyprenols, mono- and sesquiterpenoids essential oils) as perspective sources of original pharmacological compounds.

🕮 HOMOBRASSINOLIDE GROWTH-REGULATING PROPERTIES AT THE EXAMPLE OF NORWAY SPRUCE SEEDLINGS

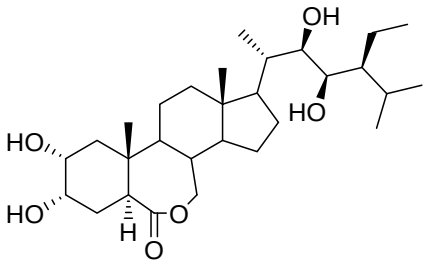
Alexey Babkov[1] and Margarita Zavadskaya[2]

[1] V.F.Kuprevich Institute of Experimental Botany of the National Academy of Sciences of Belarus, Minsk, Republic of Belarus; [2] Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus, Minsk, Republic of Belarus
e-mail: balexio@gmail.com

It is known that phytohormones such as brassinosteroids (epibrassinolide, homobrassinolide, etc.) have high biological activity, which is manifested in the regulation of plant growth and development. Brassinosteroids increase crop yields, improve the structure and quality of the crop as well as the resistance of plants to pathogens and unfavourable environmental conditions.

In the present work we studied the action of Epin plus on Norway spruce seedlings at different soil and environmental conditions in nursery garden in Belarus. The preparation Epin plus is a solution of homobrassinolide (0,25 g/l) supplemented with nonionic surfactants for better wetting by spraying of the plants.

The aim of our research was to achieve shortening of seedlings growing in the compacted school and to obtain the maximum number of standard planting material per unit area and the increase of biometric parameters of seedlings. The studies found that the double foliar treatment by Epin Plus in the dose of 60 ml/ha results in a reduction to 1 year cultivation planting material spruce, a significant increase of biometric indicators to 27,0 % and biomass of growing plants up to 64,8 %, and as a consequence increasing the yield of standard seedlings per unit area up to 15,8 %.



Homobrassinolide

The use of a large-sized planting material contributes to the rapid growth of cultures and gives them an advantage in the competition for light, water and nutrients. As a result, such landing plant material reduces the time of care for the crops. Created experimental planting of the forest crops with specified planting material indicates a high growth regulating ability of the preparation even after 5 years after planting.

According to the results of our study, preparation "Epin plus" is included into the "State Register of plant protection products ..." and is recommended for the use in cultivation of seedlings of Norway spruce.

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🕮 BRASSINOSTEROIDS DRAMATICALLY STIMULATE GROWTH AND DEVELOPMENT OF PHALAENOPSIS PROTOCORM-LIKE BODIES IN VITRO

Maryia Charnysh[1], Andrei V. Batuleu[1], and Vadim Demidchik[1*]

[1]Belarusian State University, Minsk, Belarus
e-mail: dzemidchyk@bsu.by

The content of certain phytohormones and their concentrations in a medium is the determining factor for controlling growth and differentiation of plant cultured *in vitro*. The most commonly used hormones are auxins and cytokinins. Recent studies showed that brassinosteroids (BRs) have a strong modifying effect on growth, development, sex determination and reproduction in higher plants. However these hormones are not studied for their action on growth of plant *in vitro* cultures. Moreover their effects are not investigated in such an important plant as orchids.

The aim of this work was to determine the effect of six different BRs, belonging to two main BR classes, on growth rate and development of *Phalaenopsis* × hybridum Blume protocorm-like bodies. 10^{-10}-10^{-6} M brassinolide (BL), castasterone (CS), epicastasterone (EC), homocastasterone (GC), epibrassinolide (EB) and homobrassinolide (GB) were tested. Culture of protocorms was generated from seeds of *Phalaenopsis* × hybridum Blume. Protocorm-like bodies were isolated from the primary culture and transferred to media containing various levels of BRs. Weigh and length of the protocorm-like bodies were measured after 100 days of cultivation on BR-containing media. Our data demonstrated that all BRs significantly stimulated orchid growth *in vitro*. The greatest effect on length was caused by CS while maximal increase of weight was induced by BL and EB. Orchid microclones, grown in the presence of 10^{-6} M CS, had twice bigger length that control plants. Weight gain also increased 2 and 3.5 times when plants were cultivated on media containing 10^{-8} M and 10^{-6} M BL, respectively. GB and GC caused smallest effects on growth among all tested BRs. We also compared the BR effects with classical auxins, such as indol-3-acetic acid, indole-3-butyric acid and 2,4-dichlorophenoxyacetic acid. We have found that auxins were less effective than BRs.

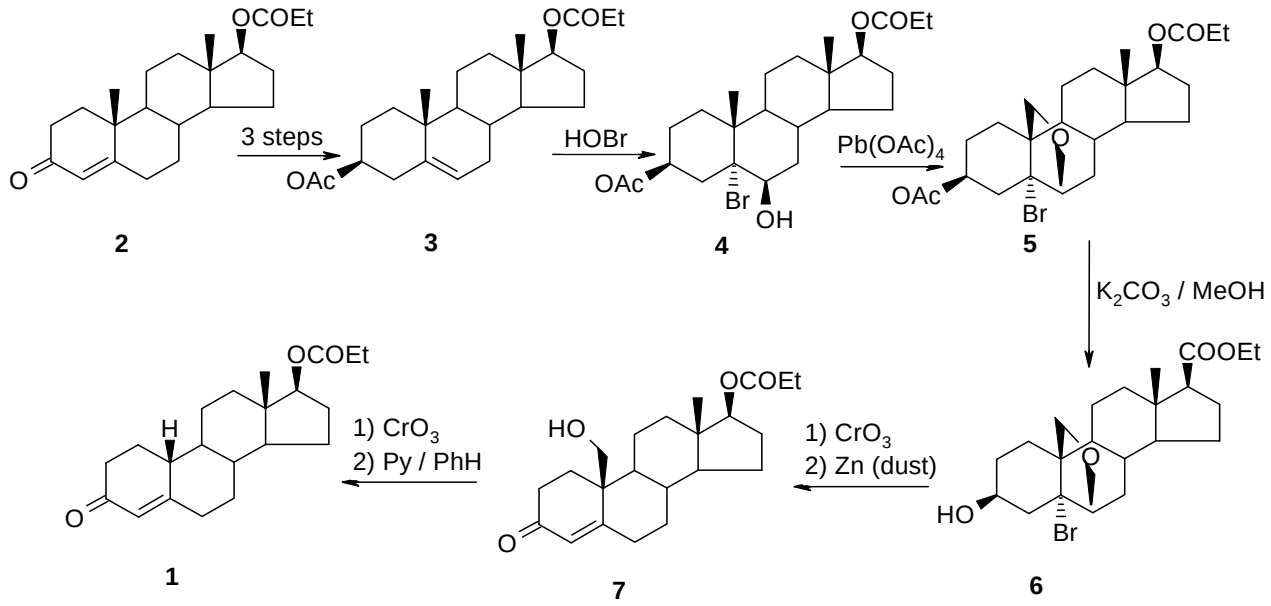
Overall, we have demonstrated for the first time that BRs stimulate growth of *Phalaenopsis* × hybridum Blume protocorm-like bodies and that this stimulation exceeds effect of auxins.

🕮 SYNTHESIS OF 19-NORTESTOSTERONE PROPIONATE FROM TESTOSTERONE PROPIONATE

Nickolai V. Kauhanka,* Vladimir I. Dolgopalets, and Yury G. Charnou

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus
e-mail: kovganko@iboch-bas.net.by

19-Norandrostane steroids are an important group of biologically active substances. These compounds are used in medicine as pharmaceuticals. 19-Nortestosterone propionate **1** is a representative example of this class of steroids. Synthetic approaches to 19-nortestosterone suffers from several drawbacks, which include low selectivity of the acylation process and the need to protect pre-3-keto group. The most convenient way to convert androstane steroids to estranes is hydroxylation of 19-methyl group, oxidation of the resulting product to 19-carboxyl synthetic intermediate and its decarboxylation[1]. This approach was previously used in the synthesis of 19-hydroxytestosterone[2], and 19-hydroxy-progesterone[3].



Synthesis of androgen 19-nortestosterones via new synthetic scheme has been achieved[4]. The synthesis commenced with commercially available **2** which was transformed to diester **3** in 3 steps. Addition of hypobromous acid to the 5(6)-double bond of the latter compound lead to bromohydrine **4** that was oxidized by lead tetraacetate to give cyclic ether **5**. Selective hydrolysis of the acetate group of the latter compound provided alcohol **6**. Oxidation of its 3β-hydroxyl group by chromic acid followed by reduction of the resulting compound by zinc dust in methylethylketone gave **7**. Oxidation of the resulting 19-hydroxysteroid by chromic acid and subsequent decarboxylation of 19-carboxylic acid by its heating in a mixture of pyridine and benzene led to the target 19-nortestosterone propionate **1**.

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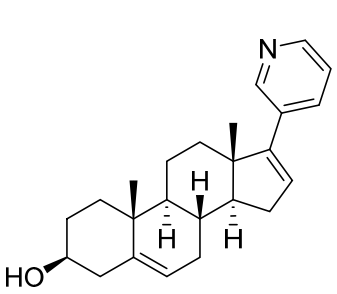
🕮 SYNTHETIC STUDIES TOWARD 17-HETEROCYCLYLMETHYLENE STEROIDS

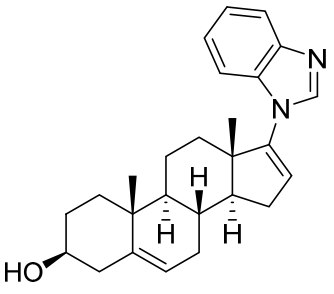
Tatsiana S. Dalidovich, Alaksiej L. Hurski,* and Vladimir N. Zhabinskii

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus
e-mail: AHurski@iboch.bas-net.by

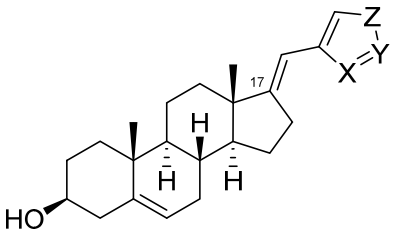
Androgens are growth factors for approximately 80 percent of all prostate cancers. CYP-17 enzymes catalyze the last step of androgens biosynthesis, and their inhibitors are used as drugs for treatment of prostate cancers. Representative examples of CYP-17 inhibitors are steroidal drugs abiraterone (**2**)[2] and galaterone (**2**).[3] Abiraterone(**1**) was approved by FDA in 2011, and galaterone(**2**) is undergoing phase III clinical trial. It is expected that steroids **3** with 17-heterocyclylmethylene moiety would also inhibit CYP-17 enzymes, and in this poster synthetic studies toward such steroids will be presented.



Abiraterone (**1**)

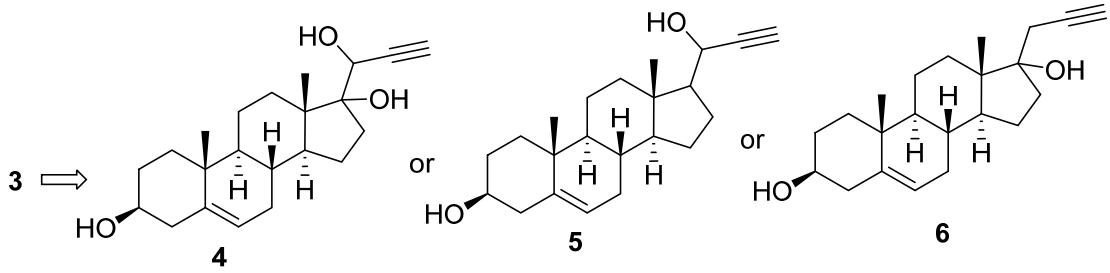


Galaterone (**2**)



3

Synthesis of heterocyclic fragment of the target molecules **3** was planned through [3+2] cycloaddition of nitrile oxides or azides to alkynyl fragments of steroidal intermediates **4**, **5** or **6**. Δ^{17}-double bond of **3** was thought to be built via Corey-Winter elimination of diol **4** or by elimination of hydroxyl groups of intermediates **5** and **6**.



4 **5** **6**

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🕮 QUANTUM CHEMICAL DESIGNING OF CHOLESTEROL AND ESTRONE CONTAINING RADIONUCLIDE CANCER-FIGHTING AGENTS

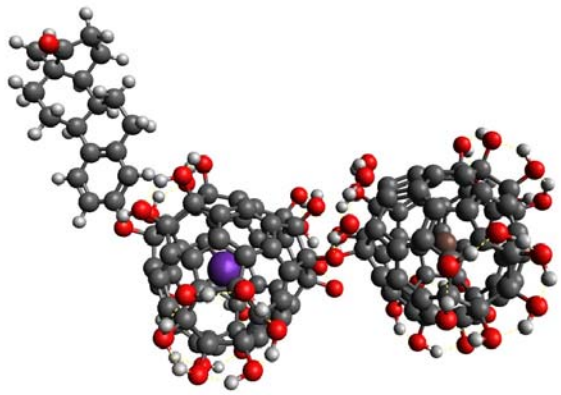
Evgenij A. Dikusar[1*], Alexandr L. Pushkarchuk[1], Dmitrij A. Rudakov[1], Vladimir I. Potkin[1], Nikolas G. Kozlov[1], Sergej J. Kilin[2], Andrej G. Soldatov[3], and Semen A. Kuten[4]

[1] Institute of Physical Organic Chemistry, NASB, Minsk, Belarus, [2] The B.I. Stepanov Institute of Physic, NASB, Minsk, Belarus, [3] The Scientific and Practical Materials Research Center, NASB, Minsk, Belarus, [4] Research Institute for Nuclear Problems of Belarusian State University, Minsk, Belarus
e-mail: dikusar@ifoch.bas-net.by

One of the commonly used cancer therapy method consists in the tumor cells irradiation by high energy proton or neutron dose to a well-defined target with little or no fractionation.

Nowadays the new modern technologies of tumor treatments are intensively introduced in clinical practice of radiation oncology: radiological destroying tumors by introducing the relevant short-lived radionuclides (Y^{90}, Zr^{95}, Fe^{59}, In^{114*}, Eu^{147}, Eu^{148}, Eu^{155}, Tm^{170}, Re^{188}, Po^{210}, Rn^{222}, U^{230}, Pu^{237}, Cm^{240}, Cm^{241}, Es^{253}), which are used in the isotope medicine. The binary (or neutron capture) therapy is a technology that was designed for the selective action on malignant neoplasm. It uses the tumor-tropic drugs that contain stable nuclides (B^{10}, Cd^{113}, Gd^{157}, etc.). The triadic therapy is a consistent placement in the body a combination of two or more tumor-tropic tissue components that are individually inactive and harmless. These components are able to be selectively accumulated in tu-

mors. They join each other in chemical interaction and destroy tumor neoplasm under certain sensitizing external influences. The designing and synthesis of new boron-organic compounds that can be used in the binary oncological diseases therapy is very perspective. In order to avoid the biological incompatibility these compounds should contain some natural isoprenoid fragments, for example cholesterol or estrone:



In order to estimate stability of isomeric cholesterol or estrone ester of *o*-, *m*-, and *p*-caboran-*C*-caboxylic acids and to study their electronic structure and construction non-empiric quantum chemical calculations of these compounds were carried out by the DFT method on the level of the B3LYP/MIDI theory following the GAMESS software.

Modelling of the electronic structure and construction of endohedral fullerenol clusters $Z@C_{60}(OH)_{23}O$-*cholesterol* or -*estron* was performed for Z = Fe, Y, Re, Po, Rn, 'empty' fullerenol cluster – monoester of fullerenol and cholesterol or estrone, and isolated atoms – incorporated components: Fe, Y, Re, Po, Rn. The necessity of preliminary investigations by computer modeling is caused by the intensive labor and high cost.[1-3]

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🕮 CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM AUTUMNAL CARPATHIAN WALNUT LEAVES

Nina Djapic[1*] and Mihailo Ristic[2]

[1] Technical Faculty "Mihajlo Pupin", University of Novi Sad, Djure Djakovica bb, 23000 Zrenjanin, Serbia; [2] Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia
e-mail: nidjapic@gmail.com

Carpathian walnut tree (*Juglans regia*, Juglandaceae) is native to the south of the Balkan Peninsula and widen all over the mainland due to the suitable soil and climatic conditions for its growth and development. Carpathian walnut brings leaves in mid-spring and defoliation occurs in early autumn. In autumn, yellow and brown Carpathian walnut leaves are gathered and it can be estimated that hundreds of tons of such leaves are left to decompose.

Yellow Carpathian walnut leaves were collected and essential oils were obtained by hydrodistilla-

tion. Qualitative and quantitative analysis were done in order to gain insight into the chemical composition. The autumnal essential oil of Carpathian walnut was rich in oxygenated sesquiterpenes (88.23%). Oxygenated monoterpenes represented 5.85%. Sesquiterpene hydrocarbons presented 1.47% of the essential oil, while monoterpene hydrocarbons were not identified. The explanation for such predominance of oxygenated sesquiterpenes is most probably in enzymes that are activated during the leaf senescence and programmed cell death.

Apart from terpenes in the essential oil identified was the aromatic compound – eugenol (1.76%). Esters were present in a quantity of 0.23%.

Autumnal leaf Carpathian walnut essential oil contained phytol (0.16%) and in green leaves, up to now, it was not identified [1,2]. There are no written reports on the presence of vitamin K_1 and vitamin E in Carpathian walnut leaves. For the timebeing, it will be estimated that the phytol quantity registered in autumnal leaves refers to chlorophyll and its catabolism.

The Carpathian walnut autumnal leaves can be used as a source for the isolation of a few oxygenated terpenoids, those most abundant in the essential oil.

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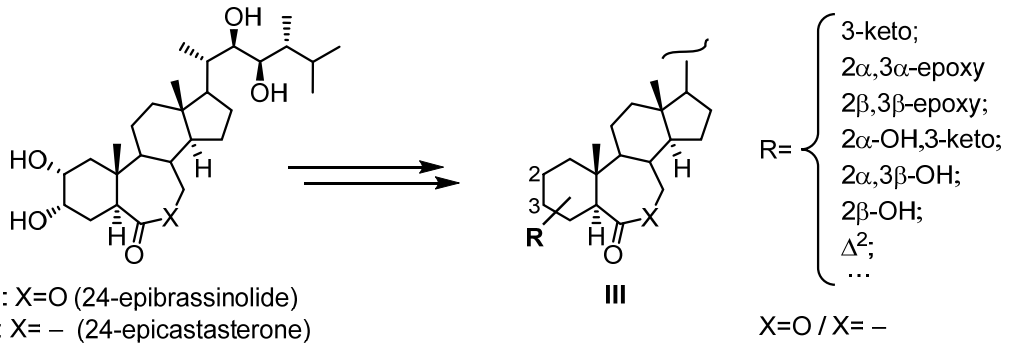
🕮 24-EPICASTASTERONE AND 24-EPIBRASSINOLIDE AS STARTING MATERIALS: A SIMPLE WAY TO MINOR BRASSINOSTEROIDS

Darya Osiyuk, Yuri Ermolovich*, Vladimir N. Zhabinskii, and Vladimir A. Khripach

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich str. 5/2, 220141 Minsk, Belarus
e-mail: ermolovich@iboch.bas-net.by

Practical use of some brassinosteroids (e.g. epibrassinolide) in agriculture resulted in the reduction of their price and made it possible to utilize them as starting materials for the preparation of less available steroids of this class. Recently, the strategy based on the one-pot transformation of epibrassinolide **I** or epicastasterone **II** to the corresponding 22-aldehyde, followed by its successive coupling with synthon of appropriate side chain proved to be quite effective in the synthesis of a sufficient number of minor brassinosteroids.[1-3]

As far as A-ring functionalization of minor brassinosteroids and commercially available starting **I**, **II** is often non-identical, the development of simple and efficient methods for the transformation of 2α,3α-diol function into the moieties, typical of the target compounds, is an important problem to be solved. In this work, syntheses of minor brassinosteroids based on the modifications of A-ring of **I** and **II** will be discussed.



I: X=O (24-epibrassinolide)
II: X= – (24-epicastasterone)

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🕮 EFFECT OF 24-EPIBRASSINOLIDE ON THE LIPID COMPOSITION IN DETACHED LEAVES OF *PISUM SATIVUM* L.

Evgenia Fedina*, Andrey Yarin, Faimya Mukhitova, Alexander Blufard, and Ivan Chechetkin

Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Sciences, Kazan, Russian Federation
e-mail: lightsolo@yandex.ru

Brassinosteroids (BRs) have recently been considered as stress hormones since their level increases under adverse environmental conditions and that leads to improvement of plant resistance.[1] Plants deficient in the enzymes participating in BR biosynthesis and perception have a phenotype of prolonged leaf lifespan and so they are notable for the delayed senescence.[2] BRs also were shown to promote chlorophyll degradation and decrease anthocyanin content in leaves.[3] Based on these observations, BRs are thought to regulate the plant senescence processes.[4-5] Nevertheless, mechanisms underlying the regulation of plant senescence by BRs need to be revealed.

Plant leaf senescence is a complex process of the final stage of plant life, which is characterized by dramatic changes in metabolism in all cellular compartments followed by modification of macromolecules as well as lipids. Lipid metabolism in the senescent plant leaves was thoroughly studied but no data on its regulation by BRs have been reported yet. The accelerated leaf senescence is known to occur in the detached leaves and so the latter are often used as a model system for studying senescence.[6]

In the present work, the system was used to study the effect of 24-epibrassinolide (EBR), one of physiological active BRs, on the composition of some lipid classes (free fatty acids, triacylglycerols and galactolipids) in detached pea leaves.

Our data demonstrate the effect of EBR (0.1 μM) on fatty acids (FA) composition in detached pea leaves for the first time. Most changes were seen in FA content of different lipid classes. EBR increased the content of free FA as well as FA bound to triacylglycerols and decreased the content of FA bound to galactolipids. These findings suggest that FA are liberated from polar lipids and then undergo esterification to neutral lipids in the detached (senescent) leaves upon EBR treatment.

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24-Epibrassinolide was a generous gift from Prof. Vladimir Khripach (Laboratory of Steroid Chemistry, Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk). This work was supported by the Russian Foundation for Basic Research (project nos.16-04-01553).

🕮 BIOCONVERSION OF 6-(N-METHYL-N-PHENYL)-AMINOMETHYL-ANDROSTANE STEROIDS BY THE NOCARDIOFORM ACTINOBACTERIAL STRAIN

Galina Sukhodolskaya[1], Victoria Fokina[1*], Andrei Shutov[1], Vera Nikolayeva[1], Tatiana Savinova[2], Yuri Grishin[2], Alexey Kazantsev[2], Nikolay Lukashev[2], and Marina Donova[1]

[1] G.K. Skryabin Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow region, Russian Federation, [2] Faculty of Chemistry, M.V. Lomonosov Moscow State University, Moscow, Russian Federation
E-mail: vvfokina@rambler.ru

The N-methyl-N-phenyl-aminomethyl functionality is one of the most efficient substituents which significantly changes steroid features and may influence different physiological activities. Except for some aminosteroids (1), nitrogen-containing androstanes have not been strongly investigated as the substrates for bioconversion.

Nocardioform actinobacterial strain Nocardioides simplex VKM Ac-2033D is an effective microbial catalyst capable of 1-dehydrogenation of a variety of 3-ketosteroids. We report here an application of N. simplex cells for 1-dehydrogenation of newly synthesized α/β-diastereomers of 6-(N-methyl-N-phenyl)-aminomethyl-androst-4-ene-3,17-dione (6-MPhAM-AD) and 6-(N-methyl-N-phenyl)-aminomethyl-androst-4-en-17β-ol-3-one (6-MPhAM-T) in comparison with their unsubstituted analogs, - androst-4-ene-3,17-dione (AD) and androst-4-en-17β-ol-3-one (T).

N. simplex cells actively perform 1-dehydrogenation of 6-MPhAM-AD and 6-MPhAM-T as well as AD and T. 1-Dehydroderivatives were identified as major bioconversion products from all the substrates tested (Fig.1). The structures of steroids were confirmed using TLC, HPLC, MS, ^{1}H-and ^{13}C-NMR and elemental analysis data.

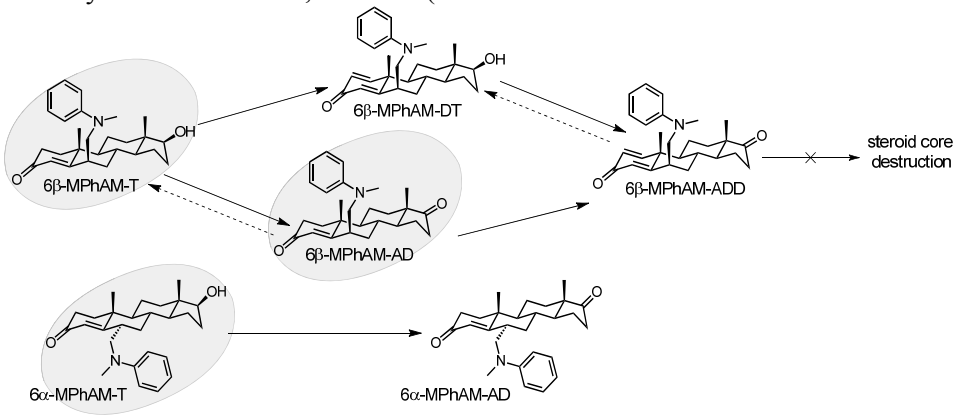

Fig.1. Bioconversion of 6α/β-MPhAM-derivatives of AD and T.

Along with 1-dehydrogenation, N. simplex oxidized hydroxyl group at C-17 of 6-MPhAM-T. Both α- and β-isomeric forms of 6-MPhAM-T were transformed to the corresponding 17-keto derivatives. In general, the rate of conversion of the unsubstituted androstanes was by over ten times higher as compared with that of their 6β-MPhAM-substituted analogs. As one of the reasons, higher hydrophobicity of the substituted substrates and its hindered transport into the cells was proposed. Application of DMSO as a co-solvent partly enhanced the conversion thus confirming this suggestion. No steroid core destruction was observed at the conversion of 6-substituted androstanes, while it was significant when the strain incubating with the unsubstituted AD and T.

The known bacterial degradative pathway proceeds via 9α-hydroxylation after 1(2)-double bond introduction into C_{19} 3,17-dioxosteroid with unstable intermediate formation followed by spontaneous cleavage of the C9–C10 bond and further degradation. In accordance with the current knowledge on

3-ketosteroid dehydrogenase (KstD) active site, the α-face of the 3-oxosteroid is in close proximity to the isoalloxazine ring of FAD (Fig.2).

1-Dehydroderivatives with an axial orientation of the substituents (6β-MPhAM-ADD and 6β-MPhAM-DT) were accumulated as major products, while no any 1-dehydrogenated α-isomers were formed. Probably, the equatorial (α) position of the bulky substituent prevents substrate interaction with KstD. Accordingly, an increase of β-stereoisomer content in the substrate α/β-stereoisomer mixtures resulted in higher yields of their 1-dehydrogenated derivatives. The lower KstD activity towards 6β-substituted androstanes in comparison with AD or T could also be explained by increased size of the complicated substituent at the axial 6β-position.

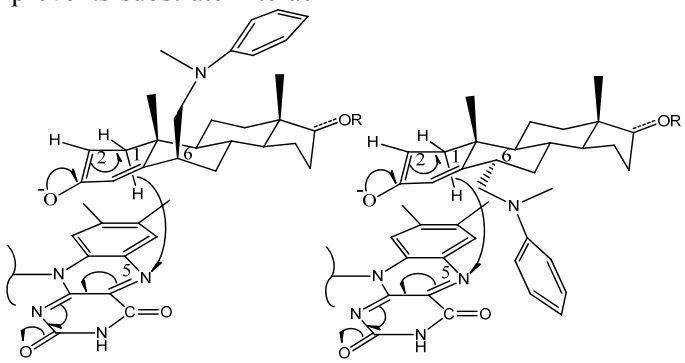

Fig. 2. Proposed effect of α/β-orientation of the (*N*-methyl-*N*-phenyl)-aminomethyl functionality at C-6 of AD or T (R=0, AD; R=H, T) on the 1-dehydrogenation mechanism catalyzed by KstD (adopted from (2)).

The permissible size was formulated earlier as no more than two carbon atoms in the chain of a β-face axial substituent (3). However, bulkier 6β-MPhAM-AD and 6β-MPhAM-T selectively converted by the strain to 6β-MPhAM-ADD. For the 6β-substituted substrates it can be proposed that this orientation of the large, partially hydrophobic and locally polar branch spatially screens the active site of the KstD (2). As shown earlier for KstD from Arthrobacter simplex, the presence of bulky substituents in the middle part of the α-face of the substrate and especially at the A-ring area may prevent 1(2)-dehydrogenation (3).

High regio-specificity of 3-ketosteroid 9α-hydroxylase (9α-KsH) as one of many Rieske oxygenases is explained by the characteristic O_2-binding to the metal center at the core of the catalytic domain (4) and accounted for by the shape of a substrate-binding pocket and a position of active site channel of the oxygenase unit of 9α-KsH (KshA), fit for the steroid substrates (5). The known KshAs amino acid residues predicted to interact with steroid substrate bound in the active site are conserved (5). So, the presence of multi-structured substituent at position C-6 (both in α- and β-orientation) appears to negatively affect the active site of the KshA in N. simplex thus preventing 9α-hydroxylation and making impossible further degradative pathways. Noteworthy, the strain did not hydrolyze, or somehow modify the (N-methyl-N-phenyl)-aminomethyl moieties at C-6, and provided only steroid core modifications at the rings A and D.

The results evidence high potential of N. simplex VKM Ac-2033D at the bioconversion of synthetic steroids.

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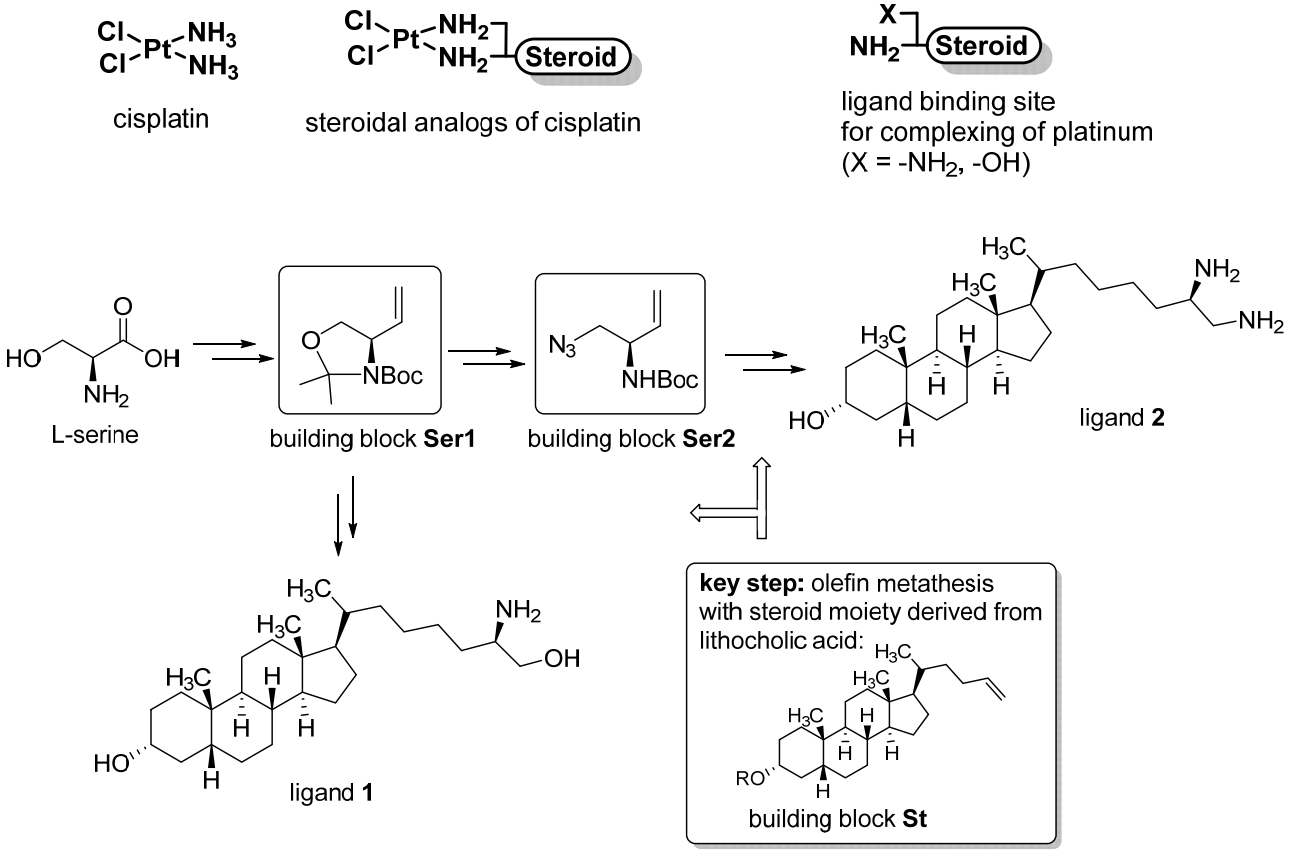
AVK, TSS, YuKG, NVL acknowledge support from M.V. Lomonosov Moscow State University Program of Development.

🕮 SYNTHESIS OF LIGANDS FOR NOVEL STEROIDAL-PLATINUM(II) HYBRID MOLECULES

Agnieszka Hryniewicka*, Jacek W. Morzycki, and Stanisław Witkowski

Institute of Chemistry, University of Bialystok, Bialystok, Poland
e-mail: aga_h@uwb.edu.pl

Cisplatin belongs to cytotoxic complexes of platinum(II) used in anticancer therapy. Unfortunately, its use is limited because of the numerous and severe side effects (nephro- and ototoxicity, low selectivity towards cancer cells) as well as tumor resistance to the drug. Therefore, new platinum complexes bearing steroid moiety were designed. Novel steroidal ligands have a binding arm capable of complexing platinum ion. It can be expected that the presence of a steroidal carrier in the structure of cytotoxic agent may cause more selective delivery of the drug to cancer cells.



Synthesis of new ligands was started from preparation of two types of building blocks ended with terminal C=C double bonds: modified L-serine[1] (**Ser1** and **Ser2**) and derivative of lithocholic acid[2] (**St**). In the next step two blocks (**Ser1** with **St** and **Ser2** with **St**) were metathetically connected using Grubbs 2nd generation catalyst. After reduction of double bond followed by deprotection of amino and hydroxy functionalities new ligands **1** and **2** were obtained.

Finally, ligands **1** and **2** will be complexed with platinum and resulting compounds will be tested on selected cancer cell lines. We hope that new complexes will be more efficient and less toxic anticancer agents than commonly applied cisplatin.

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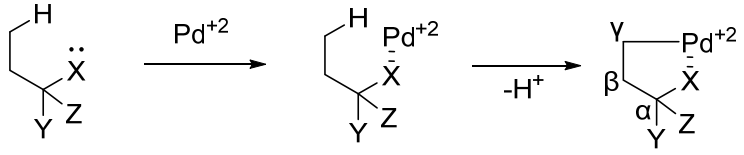
🕮 PALLADIUM-CATALYZED C-H FUNCTIONALYZATION OF 2-ALKYLSULFINYL-ANILINE AMIDES

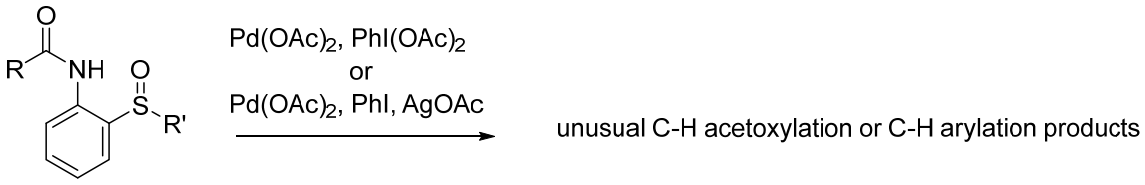
Marharyta V. Iskryk, Maryia V. Barysevich, Alaksiej L. Hurski,* Vladimir N. Zhabinskii, and Vladimir A. Khripach

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus
e-mail: AHurski@iboch.bas-net.by

Transition metal-catalyzed C-H activation reactions are powerful tool for synthetic organic chemistry[1-4] that allows direct formation of C-C bonds from unactivated C-H bonds. Such transformations may provide simple, atom and step economic pathways for making functionalized molecules.[3] The challenge of regioselective C-H activation can be addressed by incorporating a ligand directing group into the substrate undergoing functionalization. Palladium-catalyzed C-H functionalization reactions usually proceed through five membered palladocyclic intermediates, which form as a result of regioselective activation of $^{\gamma}$C-H bonds:



In this poster, we will present new directing 2-alkyksulfinylaniline groups that provide unusual palladium-catalyzed activation of $^{\beta}$C-H bonds. Presence of sulfinyl group in directing ligand is responsible for regioselectivity switching of C-H activation. The discovered directing groups were tested in acetoxylation and arylation reactions.



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🕮 TRILOBOLIDE-STEROID HYBRIDS: SYNTHESIS AND BIOLOGICAL PROFILING

Michal Jurášek[1], Petr Džubák[2*], Silvie Rimpelová[1], David Sedlák[3], Petr Konečný[2], Ivo Frydrych[2], Soňa Gurská[2], Marián Hajdúch[2], Kateřina Bogdanová[2], Milan Kolář[2], Tomáš Müller[3], Eva Kmoníčková[4,5], Tomáš Ruml[1], Juraj Harmatha[6], and Pavel Drašar[1*]

[1]University of Chemistry and Technology Prague, Faculty of Food and Biochemical Technology, CZ-166 28 Prague, Czech Republic;[2]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc, CZ-775 15 Olomouc, Czech Republic;[3]CZ-OPENSCREEN: National Infrastructure for Chemical Biology, Institute of Molecular Genetics AS CR Prague, CZ-142 20 Prague, Czech Republic;[4]Institute of Experimental Medicine, Department of Pharmacology AS CR, Prague, Czech Republic;[5]Institute of Pharmacology and Toxicology and Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Prague, Czech Republic;[6]Institute of Organic Chemistry and Biochemistry AS CR Prague, CZ-166 10 Prague, Czech Republic;
e-mail: pavel.drasar@vscht.cz and dzubakp@gmail.com.

Sesquiterpene lactone trilobolide (Tb) is a sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitor[1-2]. This contribution describes a synthesis (via click chemistry approach) and biological profiling of a series of 6 trilobolide-steroids conjugates (estradiol, pregnene, dehydroepiandrosterone, and testosterone). We observed that synthesized Tb-based compounds possess different biological activities. Cytotoxicity and preferential selectivity on cancer cells is represented by a Tb-pregnene derivative. The most cytotoxic clickates of estradiol and pregnene were studied by FACS. The impact on cell cycle and RNA synthesis was observed. Live-cell microscopy revealed the impact on cell organelle morphology particularly on endoplasmic reticulum, mitochondria and nucleus. Further, we have studied the estrogenic and androgenic properties of the clickates using cell-based luciferase assays. Finally, antimycobacterial tests showed that testosterone and estradiol derivatives potentiated the antimycobacterial activity (up to IC_{50} of 10.6 μM) in comparison to pristine Tb.

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🕮 STEROIDAL PHYTOHORMONES: REGULATION OF BIOCHEMICAL ADAPTATION OF LEGUMES

Olga Kandelinskaya[1*], Helena Grischenko[1], Valentina Domash[1], Liliya Kartizhova[2], Vladimir N. Zhabinskii[3], Olga Kosmachevskaya[4], Elvira Nassibulina[4], and Alexey Topunov[4]

[1]VF Kuprevich Institute of Experimental Botany, National Academy of Sciences of Belarus, Minsk, Belarus; [2]Institute of Microbiology, National Academy of Sciences of Belarus, Minsk, Belarus; [3]Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus; [4]AN Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia
e-mail: okandy@yandex.ru

It is known that biochemical adaptation of plants under the influence of adverse factors of the environment has as its focus the preservation of the organism integrity (homeostasis) and the functional activity of the fundamental parts of the metabolism (enantiostasis).[1] An important role in the regulation of adaptive metabolism belongs to phytohormones, in particular to brassinosteroids (BS).

It is generally recognized that BS form a group of plant hormones with a multitude of functions and perform the integrative function in plants similar to steroid hormones of animals and humans. This paradigm is fully justified by the fact that BS at extremely low concentrations (10^{-9} M or less) have a regulatory effect on all parts of the cell metabolism in plants. It starts from the reception of the hormonal signal by receptor kinases (such as leucine-rich repeat receptor-like kinases, LRR RLKs) and extends to regulation of the expression of the corresponding genes and implementing functional response.[2-5] BS were shown to be involved in regulation of hormonal status, stimulation of the nucleic acid and protein synthesis, growth and development by activating of the cell division and elongation, biosynthesis of cell wall components and seed germination, flowering, donor-acceptor relations, aging etc.[6-13]

However, there are a number of gaps in the knowledge of the mechanisms of BS action. Thus, there is still unclear the relationship between BS-induced resistance of agricultural plants to adverse effects of biotic and abiotic factors and growth processes. There is an inverse relationship in some cases between these two integral parameters of plant life. Especially it concerns the legumes whose high and stable productivity is limited by the low resistance to diseases. In addition, the adaptation processes are inevitably accompanied by temporary activation of catabolic reactions which also contributes to reduction of the agricultural product quality.

Thus, the mechanism of BS-stimulated of crop productivity and, at the same time, the threshold of sustainability to adverse environmental factors is a multi-level interconnected process that requires the detailed study for the most successful science-based of BS application in agricultural production including those oriented to organic farming.

The aim of this work was to study the effect of some BS (brassinolide, epibrassinolide, homobrassinolide) on the main elements of the metabolism of leguminous plants (the total synthesis of proteins and nucleic acids, the activity of endogenous lectins and components of proteolysis system, the functional status of symbiotic nitrogen-fixing system etc.[14-16]) and under the action of a wide range of abiotic and biotic factors such as heavy metals, fungal pathogens, insect pests.

It have been demonstrated for lupine and soybean plants that BS stimulated the biosynthesis of nucleic acids and total proteins in the leaves and maturing seeds against decrease in them the activity of a number of hydrolytic enzymes (nucleases, proteinases), the activation of trypsin inhibitors and glycoproteins of lectin family, changed the ratio of storage proteins in the seeds without compromising the quality of the essential aminoacids. The effects observed were accompanied by the activation of nodulation and nitrogen fixation in the nodules. It was also found that BS increased the metleghemoglobinreductase activity and leghemoglobin content in the nodules. The increase of bacteroide metabolic activity and levels of reducing equivalents in the cell bacteroidal culture was observed under the action of BS. It was shown that BS contribute to the reduction of toxic action of different stressors on protein metabolism, function of symbiotic nitrogen-fixing system and morphometric parameters of lupine plants.

The obtained data confirmed the idea of the authors that the growth regulating effects and protective action of BS are closely connected. In particular, the protective action is based on the ability of BS to stimulate the anabolic metabolism links[9] by temporary inhibition of the hydrolytic enzyme activity, such as nucleases and proteases in addition to the activation of proteinase inhibitors and lectins, especially under the adverse environmental conditions.

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🕮 BIO-BASED TESTOSTERONE PRODUCTION FROM PHYTOSTEROL

Mikhail Karpov[1,2]*, Galina Sukhodolskaya[1], Vera Nikolayeva[1], Victoria Fokina[1], Andrei Shutov[1], Marina Donova[1], and Nicolai Strizhov[1]

[1] G.K. Skryabin Institute of Biochemistry & Phisiology of Microorganisms, Russian Academy of Sciences, Pushchino, 142290, Russia, [2] Pushchino State Institute of Natural Sciences, Pushchino, 142290, Russia
*E-mail:*mikemikekarp@ibpm.pushchino.ru*

Testosterone (androst-4-en-17β-ol-3-one) is an important pharmaceutical androgen steroid. It plays key role in human health, well-being and sexual functioning. Currently, testosterone is produced by a four-step chemical synthesis from androst-4-ene-3,17-dione (AD).

An alternative way is via single-step biotechnological process based on microbial conversion of sterols to testosterone by using the strains capable of both sterol side-chain cleaving and 17β-reducing of 3,17-diketoandrostenes: androst-4ene-3,17-dione (AD), androst-1,4-diene-3,17-dione (ADD). However, known microbiological methods for the production of testosterone are ineffective, that is often associated with low activity of native 17β-hydroxysteroid dehydrogenase (17β-HSD) of applied strains.[1]

In this work, we used *Mycobacterium neoaurum* VKM Ac-1816D strain capable of testosterone production with an efficiency of 32% at 10 g/l phytosterol load. In order to improve its 17β-reducing activity, recombinant *M. neoaurum* VKM Ac-1816D (pNS25) strain coexpressing heterologous 17β-HSD from *Cochliobolus lunatus* and glucose-6-phosphate dehydrogenase (G6PD) from *Mycobacterium tuberculosis* H37Rv have been constructed. The presence of additional G6PD in the mycobacterial cells is necessary for the adequate NAD(P)H supply and the shifting of redox potential towards AD reduction to testosterone.

Bioconversion of 10 g/l phytosterol by recombinant mycobacteria resulted in production of 4 g/l testosterone (58% efficiency). The highest yield 6 g/l was reached with 20 g/l substrate load (43% efficiency).

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🕮 SPECIES-SPECIFICITY OF PLANT-GROWTH RESPONSE TO COMBINED APPLICATION OF N-PHOSPHONOMETHYL GLYCINE AND EPIBRASSINOLIDE

Mikalai A. Laman[1], Karina R. Kem[1], Alla F. Sudnik[1], Alina L. Sauchuk[2], and Natalia B. Khripach[2]

[1]V.F. Kuprevich Institute of Experimental Botany, National Academy of Science of Belarus, Minsk, Belarus; [2]Institute of Bioorganic Chemistry, National Academy of Science of Belarus, Minsk, Belarus

e-mail: nikolai.laman@gmail.com, kem-666@mail.ru

Recently, it has been shown that treatment of winter rape by epibrassinolide (EBl) combined with fungicides leads to decreased toxic effect of the latter ones on the plant growth.[1] This prompted us the idea to study the effects of EBl in combined application with nonselective herbicide N-phosphonomethyl glycine (glyphosate).

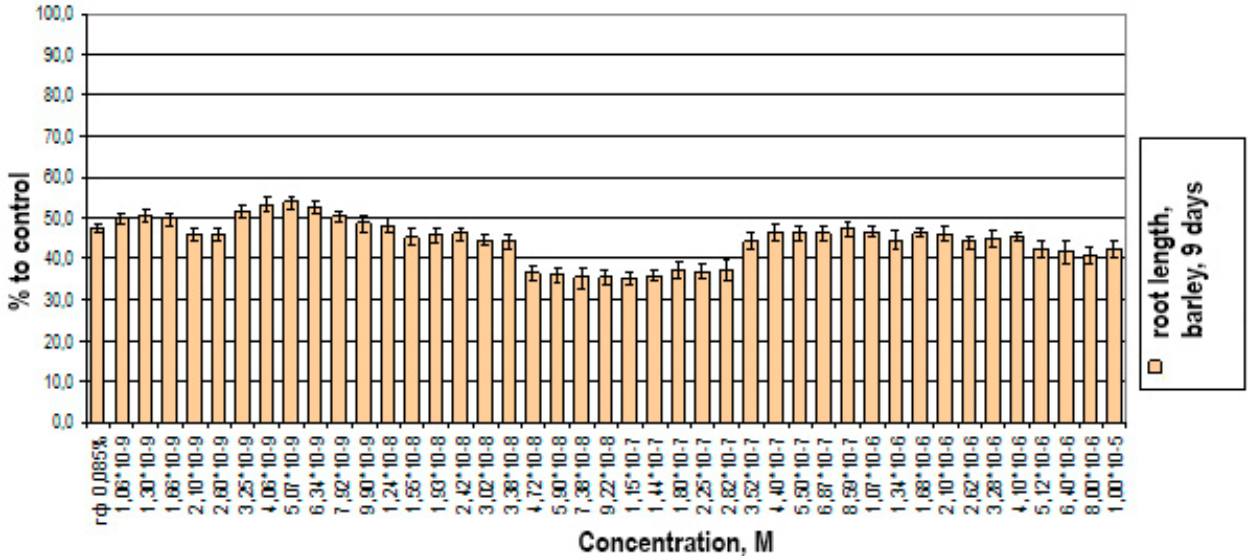

Fig. 1. Root length of seedlings of summer barley cv. Radzimich (% of control, control - 1% water solution of film-forming material) depending on the concentration of EBl under constant concentration of glyphosate (0.085%).

Seedlings of fibre flax *Linum usitatissimum* L. (cvs. Laska and Vesta) and summer barley *Hordeum vulgare* L. (cv. Radzimich) were used as model plants. Working solutions for seed treatment were prepared in a concentration range of 10^{-5}M - 10^{-9}M by dilution of basic water EBl-solution by 1% water solution of film-forming material with a single-step of 1.25 times (40 variants). It was supposed that the glyphosate concentration, which could be the most informative for investigation of its effect in a combination with epibrassinolide, was those producing 40-60% of seedlings' root-growth inhibition in comparison with the control (1% water solution of film-forming material). For the case of fibre flax of Laska cultivar it was $3.3*10^{-2}$M (0.055%), for summer barley of Radzimich cultivar and fibre flax cv. Vesta it was $5.5*10^{-2}$M (0.085%). For treatment of seeds (incrustation) 20 µl of working solution per 1 g of seeds were used. Then the seeds were germinated in paper rolls in standard conditions, and the root length of seedlings was measured at 3, 5, 7 and 9th day.

Intervals of concentrations, in which EBl influenced the effect of glyphosate, were found. For summer barley it was $4.72*10^{-8}$M – $2.82*10^{-7}$M,

and in such conditions root inhibition was about 25% higher than in the case of pure glyphosate (0.085%) (Fig. 1).

Opposite to barley, fibre flax cv. Vesta showed about 25% diminishing the growth-inhibiting effect of the herbicide in EBl-concentration range of ЭБ $9{,}2*10^{-8}$M – $6{,}9*10^{-7}$M. In cv. Laska similar but a bit lower effect (20% diminished growth inhibition by herbicide) took place in a concentration range of $5.90*10^{-8}$M – $3.52*10^{-7}$M. Measured values are statistically valid.

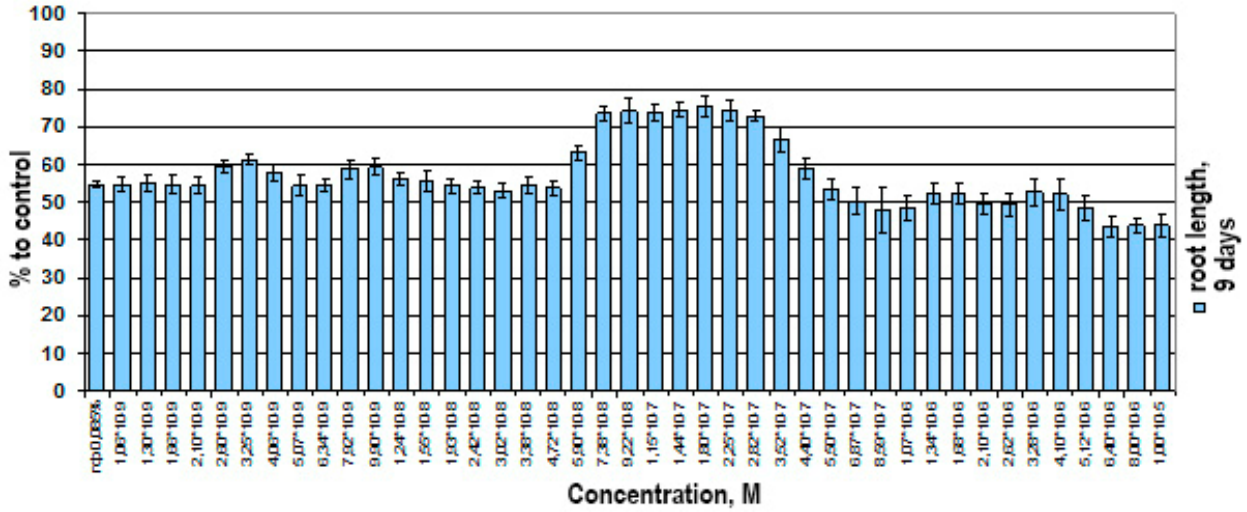

Fig 2. Root length of seedlings of fibre flax cv. Laska (% of control, control - 1% water solution of film-forming material) depending on the concentration of EBl under constant concentration of glyphosate (0.055%).

It's an interesting fact that although being oppositely directed, root-growth responses of two different plant species to the combined one-step simultaneous application of glyphosate and EBl take place in a rather narrow diapason of EBl-concentrations, which is similar for both cases.

The obtained results confirm literature data[2] on usually very narrow range of doses (or concentrations) of one of the active components of a mixture that allows to obtain maximal synergistic, antagonistic or additive resulting effect.

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🕮 OPTIMIZING XYLOSE METABOLISM FOR HETEROLOGOUS TERPENE PRODUCTION IN *E. COLI* SYSTEMS

Katarina Kemper[1], Monika Fuchs[1], and Thomas Brueck[1]

[1] Professorship of Industrial Biocatalysis, Department of Chemistry, Technical University of Munich, D-85748 Garching, Germany
e-mail: kati.kemper@tum.de

Biotechnological production of highly valuable diterpenoids, such as bioactive taxoids, using engineered *E.coli* systems is a sustainable approach for future pharmaceutical supply. While xylose is a

major component of lignocellulosic feedstocks, it cannot be metabolized efficiently by *E. coli* in the presence of glucose and other hexoses. To enable an economically sensible utilization of lignocellulosic hydrolysates for heterologous taxoid production, the xylose fraction needs to be converted efficiently by engineered *E.coli* strains. In our work we have addressed the enhancement of xylose uptake and improved carbon flux in the pentose phosphate cycle.

One prerequisite for successful heterologous terpene production in bacteria is the transformation of sugar into the metabolic intermediates glyceraldehyde-3-phosphate and pyruvate. Targeted upregulation of the intracellular concentration of diterpenoid precursors such as isopentenyldiphosphate (IPP) and dimethylallyldiphosphate (DMAPP) should thereby not affect glucose metabolism.

To achieve xylose uptake an engineered Xylose/H^+-Symporter is expressed that resolves limited xylose uptake in the presence of glucose or arabinose. Subsequent metabolomic analyses guide genomic optimization strategies to enable efficient sugar uptake and metabolism. Therefore the influence of xylose regulator XylR is tested to optimize xylose utilization. In further steps xylose usage is balanced to the need of the cell by expression of xylose isomerase and xylulose kinase. Each enzyme will be specifically controlled by selection of specific promoter systems to avoid metabolic stress.

This work is part of the SysBioTerp project, funded by the German Federal Ministry of Education and Research.

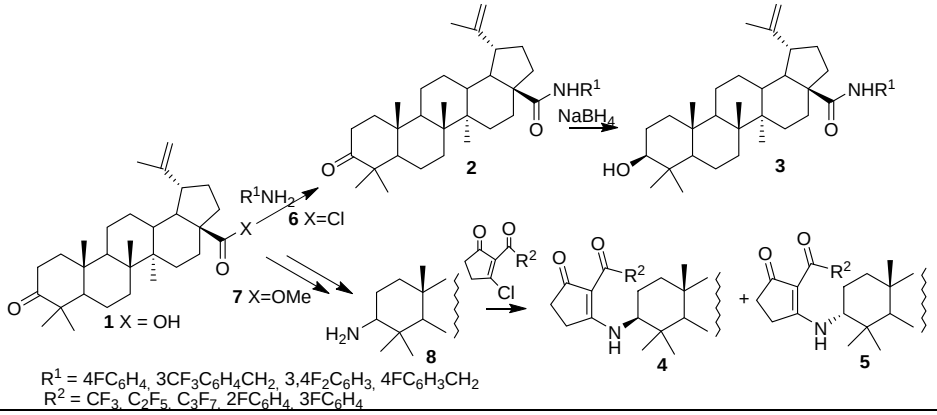
🕮 FLUORINE-CONTAINING LUPANE TRITERPENOID ACID DERIVATIVES: SYNTHESIS AND BIOLOGICAL EVALUATION AS POTENTIAL ANTI-INFLAMMATORY AGENTS

Tatyana S. Khlebnicova[1*], Yuri A. Piven[1], Fedor A. Lakhvich[1], Irina V. Sorokina[2], and Tatyana G. Tolstikova[2]

[1] Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Acad. Kuprevicha Str. 5/2, 220141 Minsk, Belarus ,[2] N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, 9 prosp. Acad. Lavrentieva, 630090 Novosibirsk, Russian Federation

e-mail: khlebnicova@iboch.bas-net.by

Lupane triterpenoids of plant origin such as betulin, betulinic and betulonic acids exhibit a variety of biological activities including antiinflammatory, antitumor, an inhibition of human immunodeficiency virus (HIV) and other activities[1]. Structural modifications based on natural triterpenes have been extensively explored to find more potent pentacyclic triterpenes as preventive and therapeutic agents. The special nature of fluorine imparts a variety of properties to certain medicines, including enhanced binding interactions, metabolic stability, membrane permeability and pharmacokinetic properties[2]. A series of fluorine-containing lupane triterpenoid acid amido derivatives **2,3** and conjugates **4,5** with fluorinated 2-acylcyclopentane-1,3-diones[3] were synthesized by



us from betulonic acid **1**.

The synthesized compounds possessed a low toxicity and displayed high anti-inflammatory activity comparable with activity of present-day nonsteroidal inflammatory drugs. It was showed on the immunogenic inflammation models that the investigated compounds suppressed an activation of cytotoxic Th1 lymphocytes and prevented developmental pseudoallergic reactions.

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🕮 THE EFFECT OF BRASSINOSTEROIDS ON ROOTING OF TREES AND SHRUBS

Dmitrii V. Kolbanov[1], Eugenia Legerova[1], Ilona Donskaya[1], Olga Saphonova[1], Tatiana Kalyaga[1], Darya Straltsova[1], Palina Chykun[1], and Vadim Demidchik[1*]

[1]Belarusian State University, Minsk, Belarus
e-mail: dzemidchyk@bsu.by

Reproduction by seeds or classical micropropagation through *in vitro* culture are not effective for most ornamental lines of woody plants, which lose their unique phenotypes when transferred to artificial conditions (*in vitro*). Therefore rooting of green cuttings is still the most widely used method for propagation of ornamental woody plants. This method has low effectiveness but allows to obtain high quality clones of original plants. Biotechnological approaches used for vegetative propagation of ornamental plants require development of new techniques for increasing rate of rooting and survival of green cuttings (shoot cuttings). To induce rhizogenes in green cuttings, ornamental horticulturists mainly use auxins, such as indole-3-butyric acid (IBA) or indole-3-acetic acid (IAA). However other hormones can also potentially be applied for rooting. Recent studies have shown that brassinosteroids (BRs) can act as synergists of auxins. Here we tested the hypothesis that these substances can also stimulate rhizogenes in trees and shrubs. We have examined the effect of epibrassinolide (EB), homobrassinolide (HB) and epicastasterone (EC) on rooting of green cuttings of *Thuja occidentalis* L. (Smaragd), *Picea abies* L. (Nidiformis), *Juniperus scopulorum* Sarg. (Blue Arrow), *Berberis thunbergii* DC (Dart`s Red Lady), *Cotoneaster lucidus* Schlecht., *Acer platanoides* L. (Drummondii) *Crataegus x media* (Paul`s Scarlet) and *Forsythia* × intermedia (*Golden Time*). We also compared effects of BRs with the action of auxins and substances with combined BR/auxin structures, such as tetraindolbrassinolide (TIBR), tetraindolcastasterone (TICS) and indolcastesterone (ICS). Obtained results have demonstrated that, in control group (treated with water), the rate of rooting was very low (10-20%). Treatment with of BRs increased rooting rate by two- to five-fold. Very similar results were obtained for auxins, however, in some cases, auxins were less effective as BRs. Response to BRs varied in different species suggesting significant complexity and evolutionary divergence in BR action on rooting of woody plants. *Picea abies* L., *Juniperus scopulorum* Sarg. and *Berberis thunbergii* DC demonstrated highest rooting rate after treatment by BRs. Intriguingly, EC was the most effective stimulator for some woody plants with much greater effect than other BRs and auxins. TIBR, TICS and ICS demonstrated less pronounced effects on rooting however they also caused some stimulation. Overall, these data demonstrated that BRs act as stimulators of formation of root system in woody plants and can be used commercially for root growth stimulation in plant biotechnology and ornamental horticulture.

🕮 NEW BIOASSAY FOR BRASSINOSTEROIDS – PEA INHIBITION BIOTEST

Petra Kořínková[1*], Jana Oklestkova, Jaromír Mikulík, and Miroslav Strnad

[1] Laboratory of Growth Regulators Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR & Palacký University Šlechtitelů 27, 78371 Olomouc, Czech Republic
e-mail: petra.korinkova@upol.cz

Brassinosteroids (BRs) represent a family of naturally occurring plant steroids that are involved in diverse developmental and physiological processes, including cell elongation, cell division, leaf senescence, vascular differentiation, flowering time control, male reproduction, photomorphogenesis and responses to biotic and abiotic stresses.[1]

Ethylene is the simplest, gaseous plant hormone composed of two carbon and four hydrogen atoms. Ethylene is produced in most plant tissues and cell types and affects a variety of processes in plants, including seed germination, growth, formation of apical hook, senescence of organ, fruit ripening, abscission, gravitropism, and stress responses.[2]

The application of low levels of ethylene to etiolated seedlings causes inhibition of stem elongation, radial swelling of the stem, and absence of normal geotropic response (aggregated apical hook), this effect is known as the „triple response".

We developed and optimized new pea inhibition test based on that BRs inhibit the growth of etiolated seedlings at high concentration; this inhibition is probably caused by ethylene production, which is mediated by BRs. We used pea seeds (*Pisum arvense L. sort Arvica*), because they grow fast and the elongation response to applied BRs is not multiphasic. We also evaluated the optimal conditions for measurement of ethylene production in etiolated pea seedlings and quantified the polar ethylene precursor 1-aminocyclopropan-1-carboxylic acid (ACC).

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🕮 SYNTHESIS OF PULEGONES AMIDES

Nikolas G. Kozlov[1*], Ludmila I. Basalaeva[1], Gayane A. Atazhanova[2], and Sergazy M. Adekenov[2]

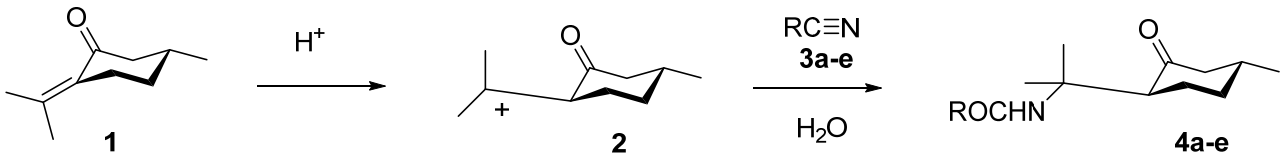
[1] Institute fiziko-organic chemistry of National academy of Sciences of Belarus 220072, Minsk, Surganov St., 13; e-mail: loc@ifoch.bas-net.by; basalaeva@ifoch.bas-net.by [2] JSC International Research and Production Holding Fitochimiya, 100009, Republic of Kazakhstan, Karaganda, Gazaliyev St. 4; e-mail: g_atazhanova@mail.ru; phyto_pio@mail.ru

Pulegone (**1**) represents monocyclic nonsaturated ketone p-mentane a row and is the main component of Ziziphora clinopodioides Lam essential oils.[1] and Mentha longifolia L.[2]

In scientific and patent literature the large number of references to the works devoted to the description of properties and biological activity of a pulegone, however about chemical modification of this monoterpenic ketone for the purpose of synthesis of nitrogen-containing derivatives is had, only two publications are found.[3,4]

Throughout our work on synthesis nitrogen-containing the pulegone[5] in the real work with us for receiving new nitrogen-containing derivatives

is carried out Ritter's reaction a pulegone with a number of both aliphatic, and aromatic nitriles (**3a-e)** (acetonitrile (**3a)**, propionitrile (**3b)**, butyronitrile (**3c)**, phenylacetonitrile (**3d)**, 1-naphthonitrile (**3e)**) in the presence of catalytic quantity the concentrated sulfuric of acid.



Obviously, process is carried out through formation of a tertiary carbocation (**2**), with the subsequent accession of a nitrile molecule to him and formation of amide. Reaction proceeds strictly stereoselective with formation of individual ketoamide with the diequatorial arrangement of metyl and izopropylamide groups. Properties of the received connection (**4a**) correspond literary for received by intermolecular C-N amination, as Ritter's reaction a mentone, N-(2-(4-methyl-2-oxocyclohexyl) propane-2-yl)acetamide.[6] Amides (**4b-e**) in literature aren't described earlier.

In Ritter's reaction important that the generated ion a carbone was rather stable as nitrile group – a bad trap for carbocation. In this work only the tertiary carbocation (2) therefore course of reaction depends on ability of a nitrile to join this ion a carbone is generated. From nine nitriles of a different structure studied by us use only four allowed to receive N-(2-(4-methyl-2-oxocyclohexyl) propane-2-yl)-R-amides. Reaction was carried out without solvent as liquid nitriles mixed up with pulegone, and crystal were dissolved in it.

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🕮 THE ANALYSIS OF VARIABILITY OF BIOPRODUCTIONAL FEATURES OF BLACK CURRANT *RIBES NIGRUM* L. IN THE PRESENCE OF PHYTOHORMONAL STEROIDS

Oksana Kudryashova[1], Galina Savchuk[1], Svetlana Stepina[1], and Anthony Volotovich[1]
[1]*Polessky State University, Pinsk, Belarus*
e-mail: volant777@tut.by

Brassinosteroids (steroid plant hormones, BS) are a promising group of natural plant growth regulators.[1] Brassinosteroids stimulate various physiological processes in plant cells, including membrane potential changes, photosynthetic and enzymatic activity, and the phytohormone balance. The effects of BS on plant growth and development show a trend of synergism with other phytohormones, auxins in particular. Regulation of plant cell growth and differentiation mediated by BS intensifies geotropic responses and accelerates stem elongation, leaf development, and pollen tube growth. It promotes xylem differentiation and pollen vitality, decelerates leaf senescence, and improves plant resistance to stresses.[1]

Investigations were conducted in the Research Laboratory of Cell Technologies in Plant Growing (Polesye State University) in 2015–2016. As objects of researches were used regenerants of Ben Alder and Tisel cultivars of black currant *in vitro*.

A subject of researches were modified by addition of steroid hormones (24-epibrassinolide, or 28-homobrassinolide) nutrient mediums on a micro, macro-salt MS-basis for whose efficiency evaluation were used the following bioproductional features at regenerant *in vitro*, such as height and number of shoots and quantity of roots. The researched concentration of phytohormonal steroids have made 0.001 mg per litter; 0.010 and 0.100 mg per litter. As additional phytohormone were used the 6-BAP in concentration of 1.0 mg per litter.

Mathematical analysis of the data (the means ± standard error, calculation of least significant differences at significance levels of $P < 0.05$ and $P < 0.01$) was performed according to standard methods of variation statistics[2] using statistics analysis software STATISTICA 6.0.[3] The dispersive analysis of data and calculation of share of factors influence on variability of the studied features carried out in the program of the statistical analysis AB-Stat 1.0 developed at Institute of Genetics and Cytology of NAS of Belarus.[4]

Usage of a nutrient medium on the MS-basis containing 0.001-0.010 mg per litter of 28- homobrassinolide leads to reliable increase in height of shoots of Ben Alder cultivar of black currant *in vitro* on average by 1.3 times, and to reliable increase by 2.6-2.9 times in quantity of roots at regenerants *in vitro* in comparison with control without hormones. In the presence of 0.1 mg per litter of 28-homobrassinolide the number of shoots at regenerants of Ben Alder cultivar of black currant *in vitro* authentically increases by 1.5 times in comparison with control without 28-homobrassinolide.

Usage of a nutrient medium on the MS-basis containing 1.0 mg per litter of 6-BAP and 0.001-0.010 mg per litter of 24-epibrassinolide leads to reliable increase in number of shoots by 2.1-2.5 times, and to reliable increase in quantity of roots by 1.4 times at regenerants of Tisel cultivar of black currant *in vitro*, in comparison with control without hormones.

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🕮 COMPLEXES OF 20-HYDROXYECDISONE WITH α-, β- AND γ-CYCLODEXTRINS

Borash I. Tuleuov[1], Tulegen M. Seilkhanov[2], Oralgazy A. Nurkenov*, Bakhtiyar S. Temirgaziyev[1], Perizat K. Kudabayeva[1], Olzhas U. Kuatbayev[1], and Sergazy M. Adekenov[1]

*[1]International research and production holding "Phytochemistry", Karaganda, Republic of Kazakhstan; [2]S. Ualikhanov Kokshetau state university, Kokshetau, Republic of Kazakhstan; *Institute of organic synthesis and coal chemistry, Karaganda, Republic of Kazakhstan.*
e-mail: phyto_pio@mail.ru

Currently for the greatest effectiveness of created pharmaceuticals the opportunities of supramolecular chemistry are used. It is known that supramolecular complexes of inclusion of the biologically active compounds (BAC) with cyclodextrins (CD) allow to regulate solubility of BAC in water, reduce their toxicity, give the chance to transfer fluid substrata into solid ones, increase stability of substances to hydrolysis and oxidation.[1-3] The possibility of creation of the nanoencapsulated complexes of the biologically active component allows not only to increase solubility and physical and chemical stability of a substratum, but also to improve its bioavailability and local acceptability. Wide use of toroidal molecules of the most widespread α-, β-and γ-cyclodextrins as host molecules is explained by the features of their structure, capacity for hydrophobic binding of guest molecules, non-toxicity and the possibility to receive it from renewable raw materials – starch.[4]

In this regard this work is devoted to research of encapsulated complexes of phytoecdysteroid of 20-hydroxyecdysone with the above-stated CD by methods of one-dimensional and two-dimensional NMR.

For the purpose of defining the type of the formed complexes of inclusion of ecdysterone with cyclodextrins the values of chemical shifts ^{1}H of a substratum and receptors in the free state and as a part of a supramolecule (tab.) were studied.

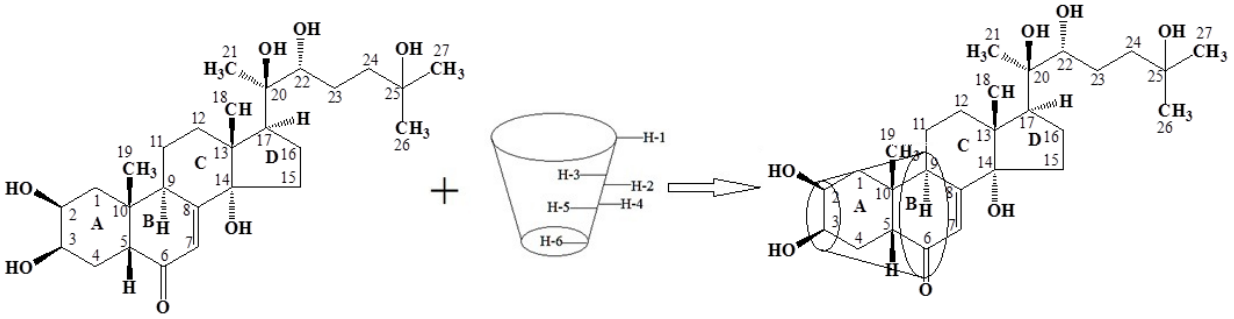
Table Values of chemical shifts ^{1}H α-, β-and γ-CD in the free state (δ0, ppm) and as part of complexes (δ, ppm)

№	α-CD			β-CD			γ-CD		
	δ_0	δ	Δ δ	δ_0	δ	Δ δ	δ_0	δ	Δ δ
H-1	4.76	4.75	–0.01	4.77	4.78	0.01	4.83	4.83	0
H-2	3.22	3.24	0.02	3.27	3.28	0.01	3.30	3.32	0.02
H-3	3.37	3.31	–0.06	3.45	3.36	–0.09	3.37	3.41	0.04
H-4	3.24	3.26	0.02	3.30	3.31	0.01	3.32	3.32	0
H-5	3.34	3.37	–0.03	3.45	3.36	–0.09	3.37	3.41	0.04
H-6	3.60	3.60	0	3.57	3.59	0.02	3.58	3.57	–0.01

NMR spectrums of initial compounds and the received supramolecular complexes were registered in a 5-mm ampoule at ambient temperature on a high resolution nuclear magnetic resonance spectrometer JNN-ECA 400 by "Jeol". Working frequency of the spectrometer is 399.78 and 100.53 MHz for ^{1}H and ^{13}C nuclei respectively. The width of wavelength scanning made about 5000 (^{1}H) and 22000 Hz (^{13}C). As the solvent DMSO-d6 of reagent grade qualifications produced by "Sigma-Aldrich" was used. Chemical shifts were measured concerning signals of residual protons or atoms of carbon of deuterated dimethylsulfoxide.

Registration of two-dimensional spectrums of COSY, HMQC, HMBC, ROESY was carried out with use of the corresponding pulse sequences included into "Delta V4.3.6" software suite.

On the basis of the provided table data it is possible to note that the largest shift is experienced by protons of the internal sphere of cyclodextrins – H-3 and H-5. Formation of an internal complex with ecdysterone is supposed. Studying of integral intensity of signals of molecules of the guest and host allows to draw a conclusion about stoichiometric relationship 1:1. For establishment of a fragment of a molecule of the hydroxyecdysone located in the internal sphere of CD, the changes of values of chemical shift were studied. The overlap of signals of ^{1}H substratum considerably complicates the analysis of NMR data, however, it is possible to assume the following scheme of complexing:



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🕮 TIME-RESOLVED FLUOROIMMUNOASSAY FOR QUANTITATIVE DETERMINATION OF BRASSINOSTEROIDS IN PLANTS

Olga Kuprienko, Alina L. Sauchuk, Raisa P. Litvinovskaya, and Oleg Sviridov

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich str., 5/2, 220141 Minsk, Belarus
e-mail: olga_garbuz@iboch.bas-net.by

Brassinosteroids play a role of low-molecular-weight bioregulators in plants. Quantitative measurement of brassinosteroids is important for determination of their natural distribution and metabolism in plants and for their application in agriculture and medicine. Enzyme-linked immunoassay (ELISA) has been developed for the detection of brassinosteriods in plants, biopharmaceuticals and fertilizers[1].

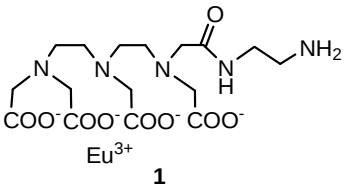
Time-resolved fluoroimmunoassay (TR-FIA) is an alternative method for quantitative determination of small biomolecules. TR-FIA is much more sensitive than other traditional fluoroimmunoassay methods. Lanthanide chelates, that are used in TR-FIA, have unique fluorescence properties which allow the measurement of specific fluorescence after the background fluorescence has already decayed. The TR-FIA of 24R-methylbrassinosteroids (24-epibrassinolide and 24-epicastasterone) has been developed for the first time.

For the syntheses of 24-epicastasterone conjugates, the derivatives of Eu^{3+} diethylenetriameneteraacetate **1** and **2**[2] have been used.

The syntheses of conjugates **4** and **5** (Scheme 1) were carried out in an aqueous dioxane solution. The reaction of Eu^{3+} tetraacetate **1** with 24-epicastasterone-6-(O-carboxymethyl)oxime N-hydroxysuccinimide ester **3** gave rise to the conjugate **4**. It was purified on TLC plate using acetonitrile/water (4/1). The conjugate **5** of 24-epicastasterone with human serum albumin (HSA) was obtained by the interaction of the activated N-hydroxysuccinimide esters **3** and **2** with HSA followed by chromatography on a Superose 12 column (1x30 cm).

In TR-FIA, immobilized antibodies against 24-epicastasterone (80-100 % cross-reactivity with 24-epibrassinolide)[1] were used.

Conjugates **4** and **5** were applied as labeled antigens. Conjugate **5** was chosen for the quantitative determination of 24R-methylbrassinosteroids because its maximum fluorescent intensity (F_0) was 5-fold higher than that of conjugate **4** (30 000 and 160 000 counts for conjugates **4** and **5**, respectively). Calibration curve (Fig. 1) was linearized by the log-logit transformation: logit F/F_0 = $\ln((F/F_0)/(100 - F/F_0))$. The limit of detection, 50% inhibition concentration value and a liner range (part of the curve between 85% and 12% F/F_0) were 0.5 nmol/L, 19.5 nmol/L and 1-1000 nmol/L, respectively.



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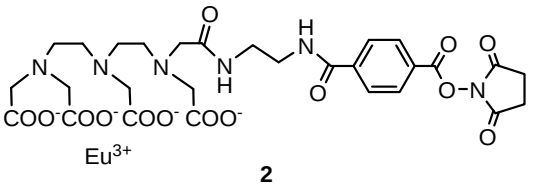

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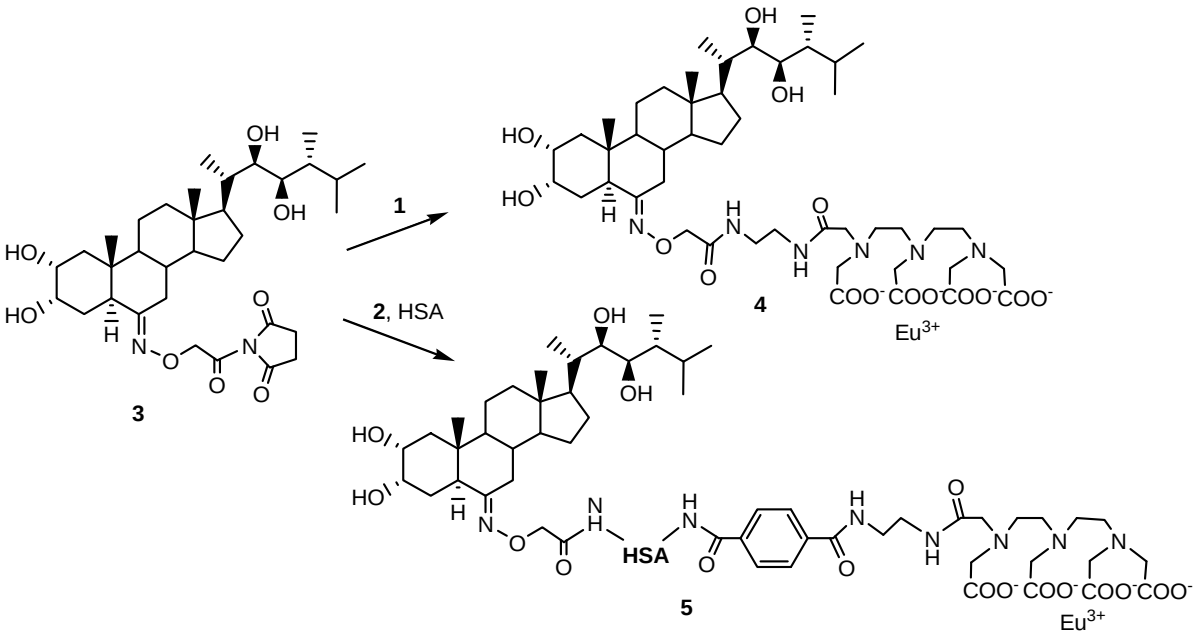

Scheme 1. Syntheses of 24-epicastasterone conjugates.

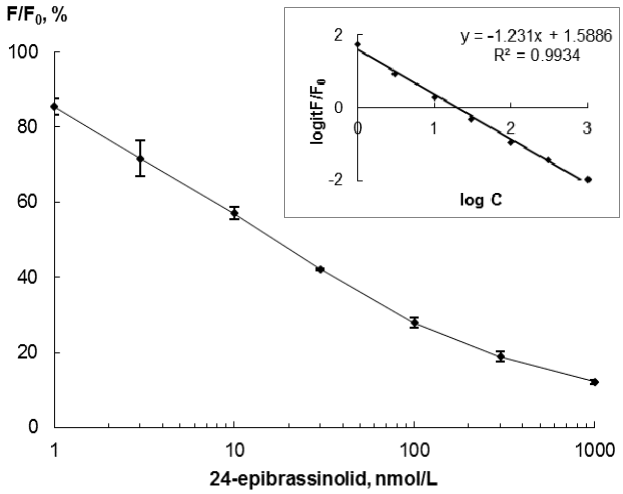

Fig. 1. Calibration curve for 24R-methylbrassinosteroids TR-FIA

Accuracy and precision of the developed TR-FIA method were studied. The level of 24R-methylbrassinosteroids in the 10-days-old seedlings of winter wheat was determined. Isolation of a brassinosteroids fraction from freeze dried plants was carried out according to the scheme presented earlier[3]. The concentration of brassinosteroids measured by TR-FIA (25.5±3.04 ng/g dry weight) was comparable with that obtained by ELISA[1] (24.9±1.56 ng/g dry weight). The recovery of 24-epibrassinolide added to plant extract ranged from 85 % to 105 %. The imprecision of the assay estimated by replicate determinations of brassinosteroids levels in 3 plant samples ranged from 4 % to 12 %. The effect of matrix was studied using serial dilution of 3 plant samples by an assay buffer. The determined concentration of 24-epibrassinolide changed linearly.

A new immunoassay for brassinosteroids has been developed, and it can be used for quantitative determination of 24R-methylbrassinosteroids in plants.

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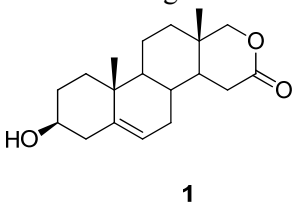
🕮 SYNTHESIS, STRUCTURAL ANALYSIS AND CYTOTOXIC PROPERTIES OF NEW HYDROXYL AND BROMO STEROIDAL 17A-LACTONES

Ivana Z. Kuzminac[1*], Dušan Škorić[1], Olivera R. Klisurić[2], Andrea R. Nikolić[1], and Marija N. Sakač[1]

[1] Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia, [2]Department of Physics, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia
[*]e-mail: ivana.kuzminac@dh.uns.ac.rs

In order to obtain potent cytotoxic compounds, derivatives of known 17a-lactone **1** were synthesized. Starting compound **1** was synthesized according to known procedure.[1] Stereospecific dihydroxylation of **1** gave 3β,5α,6β-trihydroxy derivative **2**, while 5α,6β-dibromide **3**, 5α-bromo-6β-ol **4** and 5β,6β-epoxide **5** were obtained through two synthetic steps. Furthermore deprotection 3β-hydroxy group of compound **3** gave 3β-hydroxy-5α,6β-dibromide **6**. Molecular and crystal structures were confirmed by detailed NMR (^{1}H, ^{13}C, HSQC 2D, 2D HMBC, NOE-experiments) and X-ray analysis.



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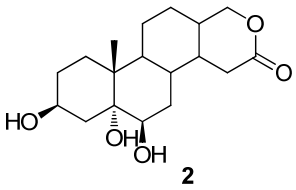

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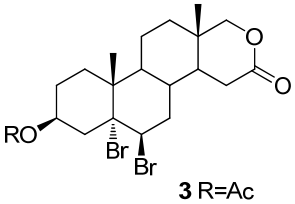
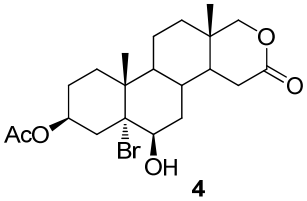


3 R=Ac
6 R=H



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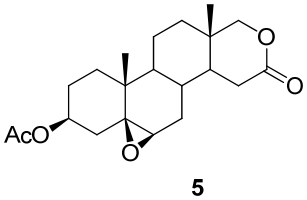

5

These compounds were tested for their cytotoxic activity against several human tumor cell lines and a healthy human cell line. In order to investigate the effect of hydroxyl group and bromine in newly synthesized molecules on cytotoxic activity, in analysis of the results have been established correlations between structure and activity of the tested compounds.

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🕮 STEROID HORMONE ANTAGONISTS. SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL PENTACYCLIC STEROIDS WITH AROMATIC A RING

Yury V. Kuznetzov[1*], Alexander M. Scherbakov[2], Irina V. Fedyushkina[3], Igor V. Zavarzin[1], and Inna S. Levina[1]

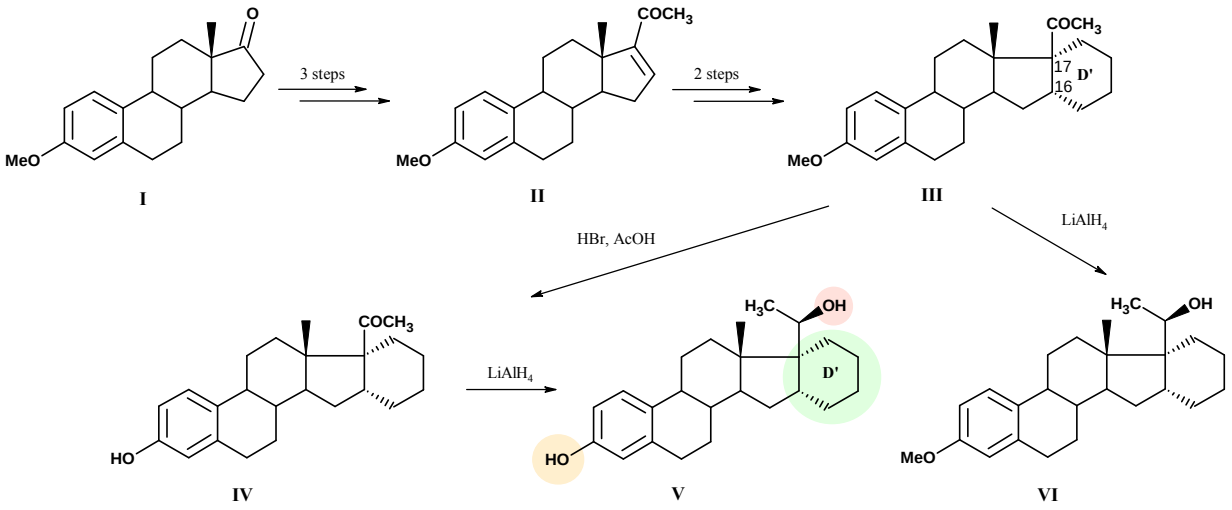
[1] Zelinsky Institute of Organic Chemistry of Russian Academy of Sciences, Leninsky pr. 47, Moscow, 119991 Russia, [2] Blokhin N. N. Russian Cancer Research Center, Kashirskoye Shosse, 24, Moscow,115478 Russia, [3] Orekhovich Institute of Biomedical Chemistry of Russian Academy of Medical Sciences, ul. Pogodinskaya 10, Moscow, 119121 Russia
e-mail: yukuv@mail.ru

Development of effective drugs for the treatment of hormone-dependent cancer remains an important trend in medicinal chemistry. So today, one area of intense research is the search for new antagonists of steroid hormones, particularly estrogens, playing a significant role in the development of breast cancer.

Further to our work on pentacyclic steroids we synthesized previously unknown steroid compounds that combine in its structure an aromatic ring A and additional cyclohexane ring D' at the 16α,17α-positions of steroid core, and examined *in vitro* their cytotoxic and antiestrogenic activities.

Firstly we performed the 3-stages conversion of 3-O-methylestrone **I** to conjugated ketone **II** which as a dienophile reacted with butadiene by $AlCl_3$-catalyzed Diels-Alder cycloaddition. After hydrogenation of the double bond in the additional D' ring appeared of the cycloadduct formed (not shown in the scheme) the key pentacyclic compound **III** was obtained. The latter was demethylated to give compound **IV** by hydrobromic acid and then reduced by lithium aluminium hydride to compound **V**. Compound **VI** was obtained by direct reduction of the keto group of **III** without demethylation.

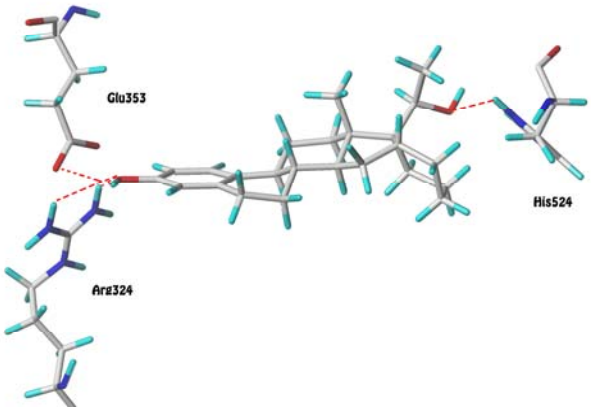
The structures of all products obtained were confirmed by ^{1}H NMR, ^{13}C NMR spectra, HRMS, and X-ray analysis.

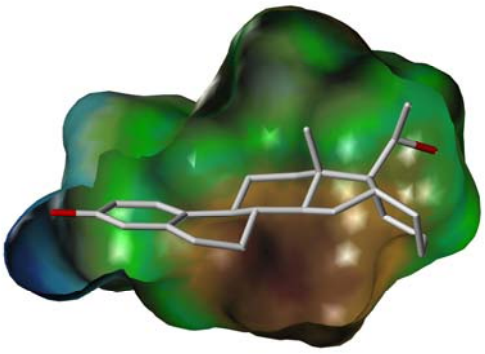


The compounds **III** - **VI** were tested *in vitro* for their cytotoxic activity against estrogen receptor-positive MCF-7 breast cancer cell line. Cytotoxicity was evaluated by the MTT test based on the accumulation of the MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) by living cells. The highest activity was displayed by the compound **V** with two hydroxyl groups (IC_{50} = 6.8 μM) and no effect was found for the compound **III**. MCF-7 cells were less sensitive to the steroid provided with 20-keto group (compound **IV,** IC_{50} = 15.8 μM). 3-Methoxy analogue of the compound **V** (**VI**) revealed no cytotoxic potency towards the MCF-7 cells. Among these novel

series only the compound **V** showed cytotoxicity that may be comparable to the effect of antiestrogen tamoxifen (IC_{50} = 5.2 μM). Estrogen receptor α (ERα) was evaluated as a key target for the obtained steroids.

Molecular docking calculations were also performed and the results demonstrated that compounds IV -VI could bind at the estrogen receptor α (ERα) with estimated binding free energies correlated well with the *in vitro* biological profiles. In particular, both 3- and 20-hydroxyl groups of the compound **V** formed hydrogen bonds with the same amino acid residues of the proteins in ligand binding domain (LBD) as estradiol (E2) in the X-ray structure of ERα-LBD E2 complex *[PDB:1QKU]*, and the molecule was well enough accommodated inside the ligand-binding pocket (Fig.).





To confirm the results of molecular modeling, the ER luciferase reporter assay was performed. The ability of compounds to inhibit 17β-estradiol-induced ER activity was analyzed in MCF-7 cells after ER luciferase reporter plasmid transfection. The β-galactosidase plasmid construct was used to normalize for transfection efficiency. Compound **III** displayed weak antiestrogenic activity when steroids **IV** and **VI** noticeably inhibited ER. The highest ER antagonistic potency was showed by the compound **V,** this chemical treatment caused 15-fold down-regulation of 17β-estradiol-induced ER activity.

Thus, the series of novel pentacyclic steroid compounds were obtained. Compound **V** with two hydroxyl groups may be considered as possible anticancer agent showing high cytotoxicity in hormone-dependent type of breast cancer. The ability of this compound to bind to ER and effectively inhibit its transcriptional activities was confirmed by molecular modeling and reporter assay.

🕮 AN IMPROVED SYNTHESIS OF 14β-METHYLISOXAZOLYL STEROIDS AND CLEAVAGE OF THEIR HETEROCYCLIC RING

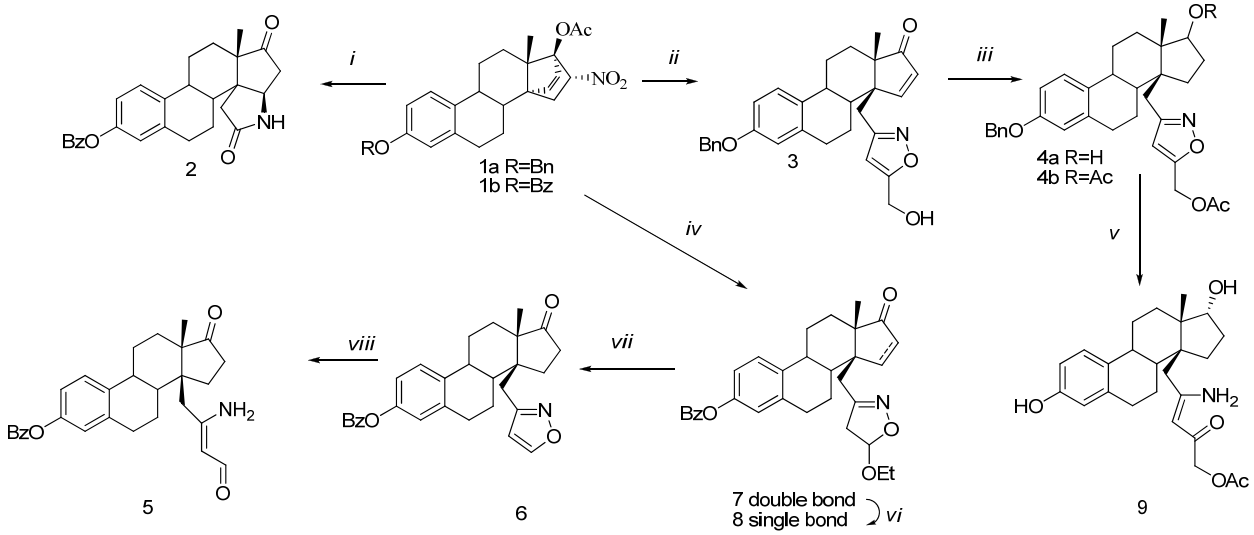
Alesia Ladyko and Alexander Baranovsky

Institute of Bioorganic Chemistry, National Academy of Sciences, Belarus
e-mail: alesyaladuka@mail.ru

Recently, the mechanism of a mild basic solvolysis of nitrosteroids **1** leading to lactam **2** was disclosed (1). The detailed knowledge of the mechanism allowed us reinvestigating the synthesis of 14β-methylisoxazole **3** and 14β-methylisoxazoline **7** (2). As result, we were able to raise substantially the yield of compounds **2**, **3**, **7**. For instance, an application of aq. i-propanol instead of aq. ethanol gave 94% of epimeric isoxazolines **7** (72% in aq. ethanol) and 90% of lactam **2** (40% in aq. ethanol). Similarly, the yield of isoxazole **3** was improved by replacement of aq. ethanol with aq. t-butanol. The reaction was finished in 69% of the product (95% based on recovered steroid **1**) in comparison

with 50% yield of **3** together with lactam **2** (*ca* 20%) in aq. ethanol.

The cleavage of the isoxazole ring in compound **3** by various reagents proceeded ambiguously giving both 14,15-fused and open chain products due to interfering of the conjugated double bond. The reduction of steroid **3** with $NaBH_4$ led to saturated 17-alcohols (17α:17β=2.5:1), which were acetylated to afford acetates **4a** and **4b**. Major 17α-epimer **4a** was hydrogenated over 5% Pd/C providing enaminoketone **10** without a protection group at C-3.



i: $NaHCO_3$, 95% aq iPrOH, Δ, 3h (90%); *ii*: propargyl alcohol, $NaHCO_3$, 95% aq tBuOH, Δ, 4-8h (69%); *iii*: 1) $NaBH_4$, THF-MeOH, 10 min, 2) Ac_2O, Py, 16h, (86%); *iv*: ethyl vinyl ether, $NaHCO_3$, 95% aq iPrOH, Δ, 4h (94%); *v*: H_2, 5% Pd/C, MeOH, 3h (80%); *vi*: HCO_2NH_4, 5% Pd/C, EtOH, 50°C, 12h (92%); *vii:* TsOH, MeCN, Δ, 3h (83%); *viii*: $Mo(CO)_6$, MeCN, H_2O, Δ, 1h (55%).

Saturation of the double bond in steroid **7** was achieved under action of ammonium formate in the presence of the catalyst yielding ketone **8** and leaving its heterocycle untouched**.** Isoxazoline **8** was readily converted into isoxazole **6** under treatment with TsOH and the heterocycle of the latter was cleaved with $Mo(CO)_6$ to afford labile aminoaldehyde **5.**

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🕮 SYNTHESIS AND SPATIAL STRUCTURE OF BROMCARBENDERIVATIVE LIMONENE

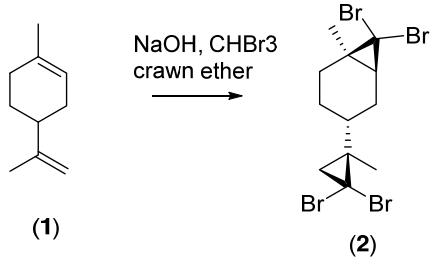
Aigerim I. Makubayeva, Gayane A. Atazhanova, and Sergazy M. Adekenov

International Research and Production Holding "Phytochemistry", Karaganda Republic of Kazakhstan
e-mail: arglabin@phyto.kz

On the base of limonene **(1)** isolated from essential oil of an *Erigeron Canadensis L.* at interaction with 50% of NaOH water solution in $CHBr_3$ in the presence of dicyclohexane-18-crown-6 for the first time new bromcarbenderivative with coming out of 20% **(2)** is synthesized. The received new derivative represents crystal-like substance of composition $C_{12}H_{16}Br_4$ with mp 144 ^{0}C dec.

The structure of the synthesized compound **(2)** is established on spectral data (Nuclear magnetic resonance ^{1}H and ^{13}C, two-dimensional spectra of the nuclear magnetic resonance ^{1}H-^{1}H (COSY, NOESY) and ^{13} C-^{1}H (COSY, COLOC)).

For final establishment of the structure of molecule is conducted its X-ray diffraction research (Fig.1). On the results of which cyclopropane units have β-conformation to carbon structure, and the methyl group at S-6 α- oriented. Isopropyl function C-3 is located in α-position, and at that C1-C2 and C1-C8 have β-orientation.



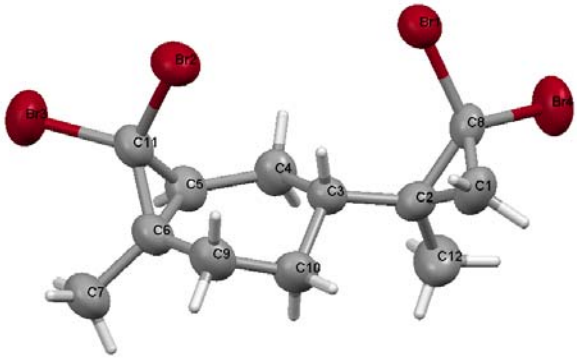


Figure 1 - Spatial structure of molecule (2) according to X-ray diffraction analysis

Thus, as a result of the carried-out work, for the first time received new derivative limonene which has structure 7,7-dibromo-4-(2,2-dibromo-1-methyl-cyclopropyl)-1-methyl-bicyclo [4.1.0] heptane, and its spatial structure is defined.

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🕮 RESTRUCTURING IN THE HORMONAL SYSTEM OF BARLEY UNDER THE INFLUENCE OF 24-EPIBRASSINOLIDE IN BIOTIC STRESS

Neli V. Manzhelesova

V.F. Kuprevich Institute of Experimental Botany at National Academy of Sciences of Belarus, Akademicheskaya str. 27, Minsk, Belarus
e-mail: manzhelesova63@mail.ru

In recent years interest researchers in the problems of induced phytoimmunity and disease resistance of plants increased. The search for safe biological preparations as an alternative to chemical ones is a basic problem in plant protection. Achievements in understanding phytoimmunity mechanisms allow development of absolutely new ecologically safe preparations, action of which against harmful organisms is realized by essential changes in host plant metabolism inducing intensification of its own protective reactions. There is a number of natural inductors of resistance among which a compound of steroid origin – plant hormone 24-epibrassinolide. Epibrassinolide is a high-effective

growth regulator. It exhibits pronounced biological activity and has a great effect on physiologo-biochemical processes of plants treated with it. It manifests itself in interaction of growth processes, morphogenesis and exerts on effect on reproductive ability of plants. At the same time, 24-epibrassinolide shows antistress, adaptogenic properties increasing plant resistance to unfavorable environmental factors; displays mediated antifungal and antibacterial activity enabling increase in non-specific resistance of plants to some pathogens. One of the fundamental properties of brassinosteroids – interaction with other phytohormones, and it is expected that brassinosteroids act as coordinators of all hormone complex. However, it is not entirely clear functional role brassinosteroids in pathosystem and how to implement their protective effect. The studies were conducted in the model pathosystem spring barley – net blotch pathogen, phytopathogenic fungus *Pyrenophora teres* Drechler. Participation of auxin in the relationship between plants and phytopathogenic fungi has long been recognized and their metabolism is given meaningful place in the theories of phytoimmunity. It is shown that *Pyrenophora teres* conidia infection of barley tissues caused a sharp reduction in the content of indolylacetic acid (IAA) in half on the second day of pathogenesis, then it gradually increased reaching the maximum on the 6th day, it dropped again up on the 10th day in comparison with healthy plants. The treatment of barley plants by 24- epibrassinolide have promoted the long time accumulation of auxin and tripled its content in one day after treatment. The same tendency observed under treatment of barley plants against infected background. Most likely that the reason of heightening of auxin level in plants treated and infected with *Pyrenophora teres* Drechler fungus is in the IAA oxidase activity inhibition. Activity of synthetase of indolylacetic acid in this conditions practically didn't change. It is expected, that caused by processing of epibrassinolide treatment increased the content of IAA in the leaf cells hampers their nekrotization. In the cell adverse conditions provided for the power supply of necrotrophic pathogen. We studied the dynamics of content abscisic acid (ABA), as a possible antagonist of the IAA in the regulation of physiological processes. However, ABA is able to activate a number of physiological processes associated with metabolic stress on inclusion of switching programs, indicating that the real opportunity to regulate the plant adaptive and protective systems not only in response to effect of abiotic factors, but also on the formation of phytopathosystems. It is shown our experiments that *Pyrenophora teres* conidia infection of barley tissues caused a marked increase in the level of ABA. Further accumulation of hormone progressively grew in intensity and on the 6th day of pathogenesis was twice higher than in healthy plants tissues. Preprocessing plants 24-epibrassinolide treatment resulted in very substantial (three times), but briefly raising the level of the hormone in the leaves of infected plants on the background. May 24-epibrassinolide provides indirect ABA effect on gene expression of PR-protein synthesis and initiates activity in the operation of the signalling system which enables protective reactions on the local and systemic level.

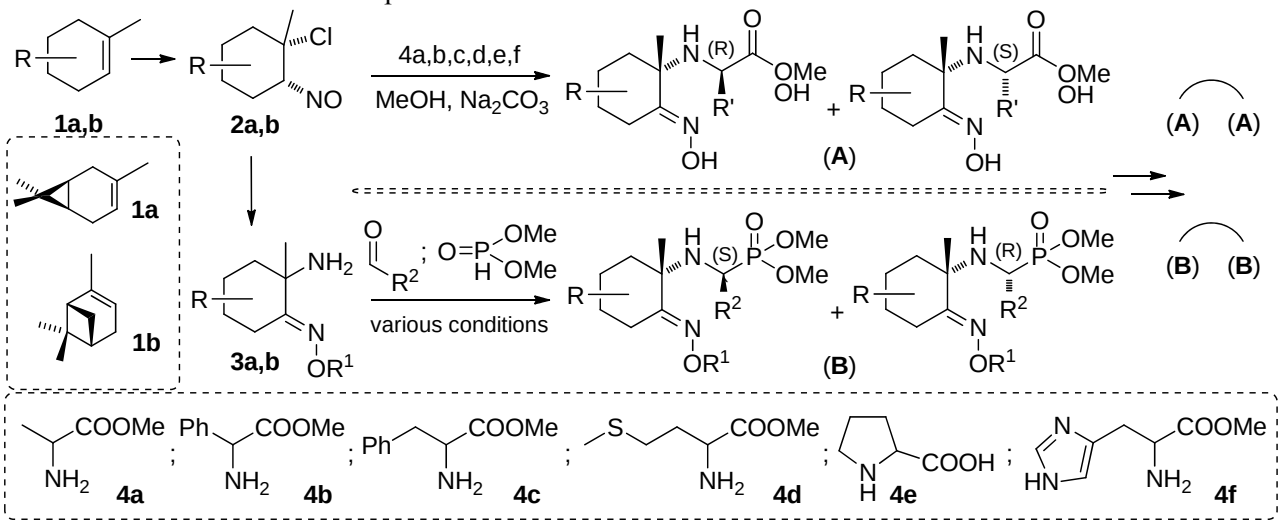
🕮 SYNTHESIS OF TERPENE-SUBSTITUTED A-AMINO ACIDS AND THEIR PHOSPHATE ANALOGUES. STEREOSELECTIVITY INVESTIGATIONS

Konstantin S. Marenin[1*], Julia A. Bryleva[2], and Aleksandr M. Agafontcev[3]

[1] N.N.Vorozhtsov Novosibirsk Institute of Organic Chemistry of Siberian Branch of RAS, Novosibirsk, Russia, [2] Nikolaev Institute of Inorganic Chemistry of Siberian Branch of RAS, Novosibirsk, Russia, [3] N.N.Vorozhtsov Novosibirsk Institute of Organic Chemistry of Siberian Branch of RAS, Novosibirsk, Russia
e-mail: demol@nioch.nsc.ru

Monoterpenes are convenient precursors for synthesis of diverse chiral compound thanks to chirality of terpenic framework and methods for their functionalization developed.[1] The terpenic-substituted α-amino acids and their phosphonic analogues are of interest as chiral ligands in vari-

ous fields[2], moreover amino-phosphonic acids found application in modern biochemistry and pharmacology[3].We investigate approaches to the synthesis of N-terpenic substituted aminoacids and their phosphonic analogues. In first case we study the reaction of nitroso chlorides **2a,b** with an amino acids or their esters **4a-f**. Yield of compounds of (**A**)-subclass varies from 15% to 87%. In experiments with racemic aminoacids it was found that the reaction in most cases is diastereoselective with the involvement of predominantly one enantiomere of amino acid or its ester. Exampled extreme ratio of (S)/(R)-amino acid containing compounds formed ranges from 1:2.7 to the yeld of (S)-aminoacid containing compounds exclusively. For the synthesis of phosphonic analogues of terpene-contained aminoacids, (**B**)-subclass compounds, we apply the Kabachnik-Fields reaction with amino oximes **3a,b** derived from monoterpens as amino component. It was found that variation of the reaction conditions and the catalyst used can change or even invert diastereomeric ratio of products.



It was demonstrated that synthesized compounds of **A** and **B** subclasses and products of theirs further functionalization are of interest as chiral ligands for synthesis of fluorescent coordination complexes.[2b,4]

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🕮 GLUTATHIONE REDOX SYSTEM MODULATION BY CALCIFEROL AND BRASSINOSTEROID ADMINISTRATION IN PREDNISOLONE-INDUCED D-VITAMIN DEFICIENCY

Andrei G. Moiseenok, Elena P. Lukienko, Valerij A. Gurynovich, Dmitrij S. Semenovich, Yurij Z. Maksimchik, and Tatiyana A. Borodina

Institute of Biochemistry of Biologically Active Compounds NAS of Belarus, Grodno, Republic of Belarus
e-mail: andrey.moiseenok@tut.by

Redox-dependent modulation of intracellular signaling cascades attracts special attention of researchers among the diverse effects of the hormonal form of vitamin D (calcitriol) in the metabolic pathways implemented by genomic and non-genomic mechanisms (eg.[1-3]). Versatility and prevalence of redox signaling can determine pathogenetic role and pleiotropic actions of vitamin D and its metabolites, in particular when initiating oxidative stress and systemic autoimmune inflammatory process. The ability to model D-vitamin deficiency by administration of prednisolone (PS), that has anti-inflammatory, immunosuppressive and anti-proliferative actions violating bone mineralization and calcium balance provides a new experimental approach of redox processes studies, which are largely mediated by glutathione (G) system.

Vitamin D-deficiency model was reproduced on adult female Vistar line rats using intragastric PS administration in a dose of 5 mg / kg for 21 days. Oil solution of cholecalciferol was selected as a source of vitamin D, it was administered at a dose of 200 IU / kg. A separate group of animals received in addition to vitamin brassinosteroid (BS) in a dose of 10 mcg / kg. Administration of PS increased catalase and superoxide dismutase activity in blood plasma, as well as the level of catalase activity and the level of thiobarbiturate-reactive compounds in the liver and kidneys, as well as reducing of the concentration of inorganic phosphorus with a tendency to reduce the overall level of calcium and the increase of Ca / P ratio in plasma. It was established that the level of cholecalciferol in the liver of animals that received PS, tends to decrease, which is eliminated by administration of vitamin D. This effect is absent when vitamin D is administered together with BS. The effectiveness of the latter does not modulate the effect of vitamin D, leading to hypocalcemia, that proves the specific effect of cholecalciferol. It was revealed that in the organs of experimental animal with intensive metabolism of cholecalciferol there are marked changes in redox status, which show growth of reconstituted G, fall of oxidized G, increase in the GSH / GSSG ratio and increase of the G redox potential in the liver, while in kidney changes are opposite. These changes were due to the appointment of PS, but their severity ware greater when supplemental cholecalciferol and BS ware administrated. There were no changes in G-peroxidase isoenzymes in liver, but decreased activity of selenium-dependent G-peroxidase in the kidney was effectively recovered by administration of vitamin D and in a lesser extent - in combination of vitamin D and BS. The manifestation of oxidative stress in the liver and in the kidneys was partially corrected by administration of vitamin D and slightly modulated by administration of BS.

At the same time it was found that BS modulates the effect of vitamin D on the total thiol-disulfide status of liver tissue, and especially kidney, where a significant effect of protein thiols increase with decreasing level of S-glutathionylation was revealed. It was also found that the combined administration of cholecalciferol and BS effectively modulates erythrocyte G redox potential, activates red cell G-transferase, and reduces the total antioxidant activity of blood plasma. Thus, violation of the redox processes in the PS-induced vitamin D-deficiency is accompanied by the development of oxidative stress and impaired G system and largely modulated by administration of calciferol and its combination with BS.

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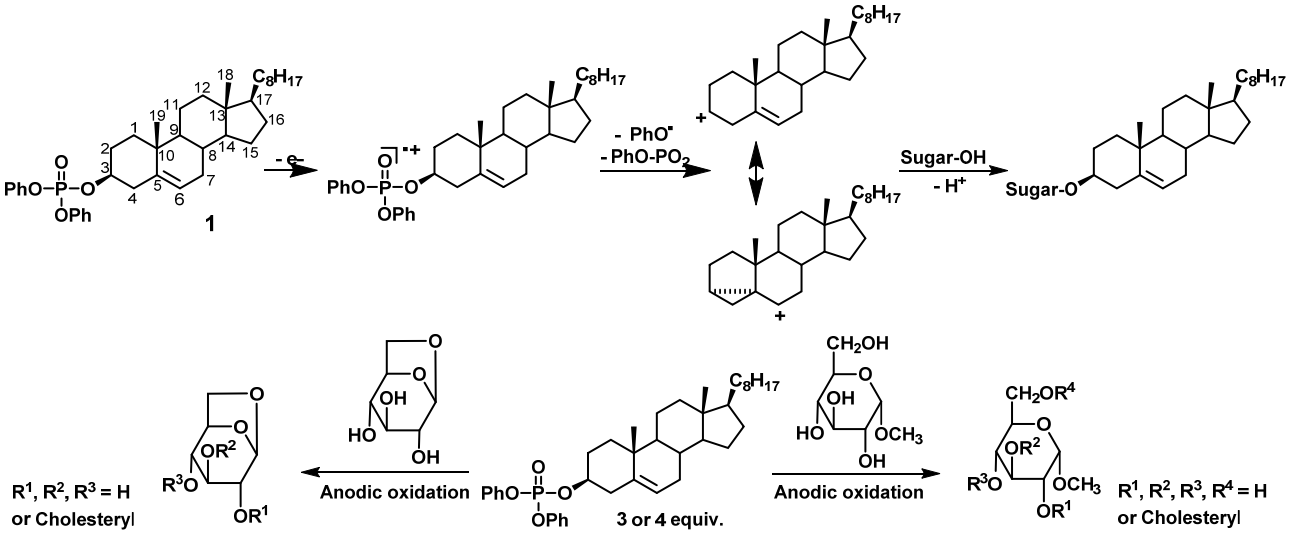
🕮 ELECTROCHEMICAL CHOLESTERYLATION OF SUGARS

Aneta M. Tomkiel[1], Jolanta Płoszyńska[2], Andrzej Sobkowiak[2], and Jacek W. Morzycki[1*]

[1] Institute of Chemistry, University of Białystok, 15-245 Białystok, Poland; [2] Faculty of Chemistry, Rzeszów University of Technology, 35-959 Rzeszów, Poland
e-mail: morzycki@uwb.edu.pl

The electrochemical oxidation of cholesterol was studied in our laboratories for several years and led to elaboration of a new method of cholesterol glycosylation. Electrooxidation of cholesterol strongly depends on the reaction conditions.[1] The reaction carried out in the non-polar solvent generates a cation-radical at the oxygen atom as a result of one-electron transfer. Further cleavage of the carbon-oxygen bond affords homoallylic carbocation that may react with any nucleophile present in the reaction mixture, including sugar hydroxyl groups.[2] The method allows to obtain glycosyl derivatives of cholesterol and other Δ^5-sterols using non-activated sugars. However, yields are usually poor and glycosylation is accompanied by side reactions of the carbocation (e.g. with cholesterol). Since cholesterol is competing with sugar for access to carbocation, dicholesteryl ether is formed as a by-product. An improvement of the glycosylation method has now been proposed.[3,4] Cholesteryl diphenylphosphate was selected as the best cholesteryl donor for electrochemical sugar cholesterylation. With this protocol only equivalent amounts of cholesterylating agent are required. Moreover, by-products are formed to a lesser extent than in previous experiments.



The reactions turned out to be completely stereoselective with respect to both sugar and steroid moieties. The original configurations at sugar stereogenic centers were retained. Only sugar 3β-cholesteryl derivatives were formed. Primary and anomeric hydroxyl groups in sugars were the most reactive ones, while no substantial differences in reactivity was found for different secondary hydroxyl groups. The mono-, di-, tri-, and tetra-cholesterylated sugar products were obtained.

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🕮 FLAVONOIDS – PERSPECTIVE SOURCES OF ORIGINAL DRUGS

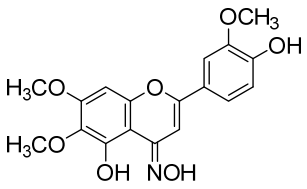
Gulim K. Mukusheva

International Research and Production Holding "Phytochemistry", Karaganda, Republic of Kazakhstan
e-mail: aligul-20@mail.ru

The study of plants on the content of polyphenolic compounds is of interest that it has an important practical value to find perspective source of biologically active substances. By now, there are more than 8000 flavonoids, for which the structure of molecules clearly established.

According to the results of the screening of enhanced study, we have identified 25 species of plants in the flora of Kazakhstan. At the same time as perspective sources of flavonoids are identified buds of Populus balsamifera L., the aerial parts of Artemisia glabella Kar.et Kir and Serratula coronate L. from which more than 20 flavonoids are identified and developed.

Efficient modification techniques practically available flavonoids cirsilineol (1), pinocembrin (2), pinostrobin (3) for a directed search of new derivatives with practically valuable properties [1-3]. Thus, new derivatives are synthesized (4-6) on the basis of molecule (1)



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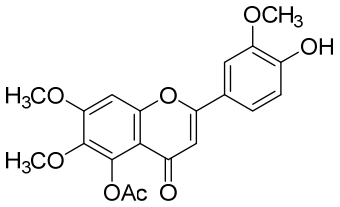

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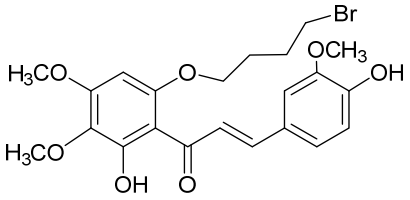

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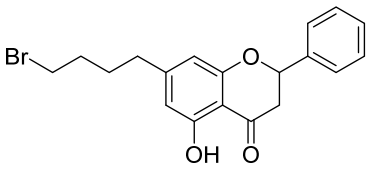

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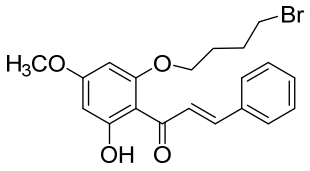
Bromination of pinocembrin (2) with 1.4 - dibromobutane leads to the formation (7). On the basis of flavonoid pinostrobin (3) was developed an original cytoprotective drug "Oxipin" in JSC IRPH "Phytochemistry". Availability of pinostrobin and its structure analysis leads to the conclusion about the perspectives of the implementation of its synthetic transformations with obtaining compounds which structurally similar to a variety of natural metabolites and other practically important substances.

In terms of obtaining new modified derivatives based on flavonoid pinostrobin, we synthesized (E) -1- (2- (4-bromobutoxy) -6-hydroxy-4-methoxyphenyl) -3-phenylprop-2-in-1-on (8), (E)-1-(2-(4-azidobutoxy)-6-hydroxy-4-methoxyphenyl)-3-phenylprop-2in-1-on (9) (E)-1-(2-hydroxy-4-methoxy-6-(4-(4-phenyl-1H-1,2,3-triazole-1-il)butoxy) phenyl)-3-phenylpropanone (10), which can be considered as potentially biologically active substances.



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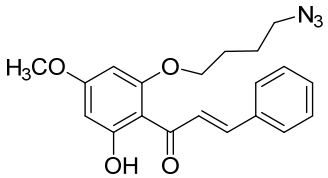

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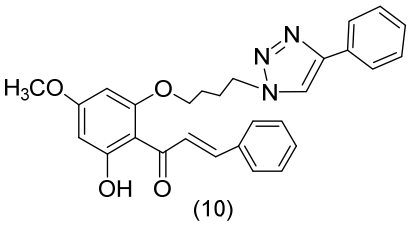

(10)

The composition and structure of the synthesized compounds were confirmed by data of IR, UV, NMR, ^{1}H-, ^{13}C - spectroscopy, including elemental analysis.

Thus, of course, further study of polyphenolic compounds from plants of the flora of Kazakhstan, including the search for new representatives of this number of compounds. The establishment of their structure, study of biogenesis, biological role and physiological activity will lead to the interesting new discoveries that finally will ensure the development of substances and pharmaceutical formulations of new drugs of herbal origin of a wide spectrum of action.

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🕮 IMMUNOSTIMULATORY PROPERTIES OF BRAVIDEFEN, A BRASSINOSTEROID-BASED DRUG, IN CHICKENS

Marina I. Likhacheva[1], Igor V. Nasonov[1*], Raisa P. Litvinovskaya[2], and Alina L. Sauchuk[2]

[1] Vyshelesski Experimental Veterinary Medicine Institute of the National Academy of Sciences of Belarus, Minsk, Belarus; e-mail address:nasonov@tut.by
[2] Institute of Bioorganic Chemistry NAS of Belarus, 220141 Minsk, Kuprevich Str.,2/5, Belarus
e-mail: alina_kyrtikova@mail.ru

The search for new high-performance products with a broad spectrum of action is one of the most urgent problems of modern veterinary medicine. Natural compounds and their derivatives such as plant hormones brassinosteroids, the first representative of which – brassinolide was extracted in 1979 by Mitchell's group from Brassica napus L. pollen, are a promising basis for the developing of new drugs[1, 2].

A new drug Bravidefen was prepared from water-soluble form of brassinosteroid 24-epibrassinolide for usage in the poultry industry as protective and stimulative medication. It was a result of joint research of the Institute of Bioorganic Chemistry and Vyshelesski Experimental Veterinary Medicine Institute of the National Academy of Sciences of Belarus.

The aim of the current research is the investigation of the immunostimulatory properties of Bravidefen in the experimental disease of chickens initiated by the Newcastle strain infection vaccine, which was administered by watering or intranasally.

To determine the immunostimulatory activity of Bravidefen three groups (2 experimental and 1 control) of one-day-old chickens were formed. Each group contained 10 chickens. Chickens of the experimental and control groups at the age of one day were intranasally immunized by КМИЭВ-V104 Newcastle disease virus strain vaccine. Bravidefen was administered during five days as follows: chickens of the 1st experimental group were watered at a dose of 1 ml (water solution of the drug with a concentration 0.25 x 10^{-4} M) per 100 g of live weight; chickens of the 2nd experi-

mental group were treated intranasally at a dose of 0.1 ml; chickens of the control group were immunized by vaccine only and did not receive Bravidefen. In the beginning (background) and at the 7, 14, 21 and 28 days of the experiment, blood samples were taken from the chickens of all groups for serological tests to determine the level of antibodies to Newcastle disease virus in the hemagglutination inhibition test (HAI).

Measuring the HAI of one-day-old chickens' blood serum (background level) showed no antibodies to the virus of Newcastle disease in all three groups. The data on serological studies of chickens' blood after vaccination are presented in Figure 1.

Figure 1 shows that in the 1st experimental group, starting from 14th day after treatment with Bravidefen, it was a significant increase in antibody titer by $2.6\log_2$ ($P < 0.05$) in comparison with the control group. At the 21st day the titer in the 1st experimental group was found as $9.4\log_2$ against $6.4\log_2$ in control ($P < 0.05$). At 28th day the quantity of antibodies in the 1st experimental group increased to $10.8\log_2$, which is in 1.38 times higher in comparison with the control group, where the titer was found as $7.8\log_2$ ($P < 0.01$).

The stimulating effect of Bravidefen on the antibody response was also observed in the 2nd experimental group. The increase of the antibodies in the chickens up to the age of 14 days did not significantly differ in this group from the control group. However, at 21st and 28th days there was a significant increase in the quantity of antibodies in 1.59 and 1.47 times ($P < 0.001$) respectively in comparison with the control group.

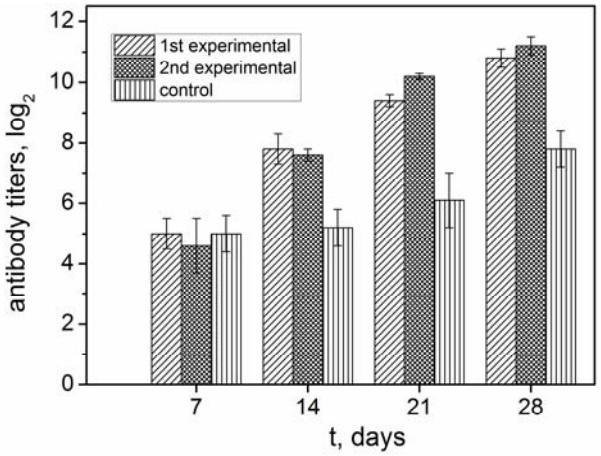

Figure 1. Bravidefen influence on chickens antibody response at Newcastle disease virus immunization

Thus, it was found that Bravidefen administered by watering or intranasally to chickens at the background of their immunization by КМИЭВ-V104 strain of Newcastle disease virus showed immunostimulatory properties, significantly increasing the production of antibodies to the virus of Newcastle disease in comparison with the chickens treated with the vaccine only.

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🕮 EFFECTS OF 24-EPIBRASSINOLIDE, 28 HOMOCASTASTERONE AND THEIR SYNTHETIC DERIVATIVES ON THE VIABILITY OF CANCER CELL LINE A549 (LUNG CARCINOMA) AND ELUCIDATION OF THE MECHANISM OF THEIR ACTION

Olesya V. Panibrat*, **Pyotr Kisselev, Aliaksei R. Sysa, and Marina V. Anisovich**

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich str., 5/2, 220141 Minsk, Belarus
e-mail: miss.panibrat@yandex.by

It is known that the treatment of cancer with classical chemotherapeutics, for example, doxorubicin, is facing a number of challenges, some of which are the side effects of their action on the body. Therefore, it becomes necessary to search for new compounds that reduce the viability of cancer cells and at the same time not having effects on normal cells. Such compounds may have a vegetable origin - brassinosteroids. Brassinosteroids have been recognized to be an integral part of the plant hormonal system.[1] Among various biological activities of brassinosteroids, the most significant one is the ability to increase plant resistance to unfavourable biotic and abiotic environmental factors. Of particular interest are numerous reports describing their anticancer properties, espessialy in respect of hormone-dependent cancer cell line.[2,3] However, very little is known about the mechanism by which they exert their cytotoxic effects.

In this work with the help of flow cytometry the effect of two natural brassinosteroids: 24-epibrassinolide, 28-homocastasterone and two their synthetic derivatives (22*S*,23*S*)-24-epibrassinolide, (22*S*,23*S*)-28-homocastasterone (Fig. 1) on the viability of hormone-independent cancer cell line A549 (lung carcinoma) was evaluated.[4] To determine the level of reactive oxygen species (ROS) was used fluorescent label 2',7'-dichlorodihydrofluorescein diacetate, and for determining the level of apoptosis and cell cycle - the intercalating dye ethidium bromide.

It has been shown that synthetic analogues of brassinosteroids increase ROS level 3-5 times, and they are more effective in reducing the viability of A549 cells, as compared to natural (Fig. 2b).

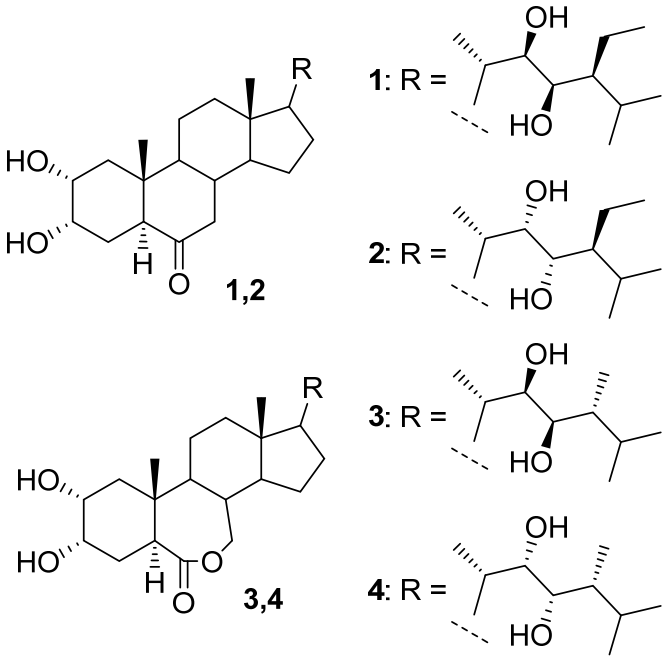

Fig. 1 Structures of evaluated brassinosteroids: 1 - 28-homocastasterone, 2 - (22*S*,23*S*)-28-homocastasterone, 3 - 24-epibrassinolide, 4 - (22*S*,23*S*)-24-epibrassinolide.

IC_{50} for (22*S*,23*S*)-24-epibrassinolide, (22*S*,23*S*)-28-homocastasterone was 100 and 130 μM, respectively (Fig. 2a). With regard to cell cycle, % S-phase under the effect of brassinosteroids reduced in some extent, but the greatest inhibition was observed under the influence of 24-epibrassinolide-28% as compared with controle -44%. At the same time, both natural compounds caused a doubling level of apoptosis compared with the control.

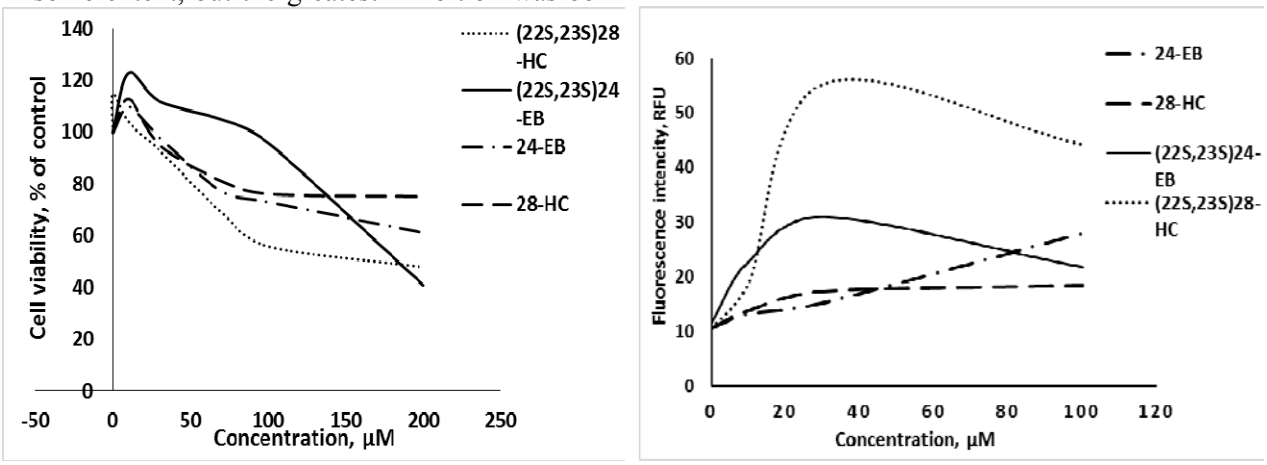

Fig. 2 – Influence of brassinosteroids on a.) cell viability; b.) intrinsic level of ROS.

For investigating possible mechanism of cell death caused by test compounds, a series of experiments using ethidium bromide (Et-Br) was conducted. It is known that ethidium bromide has intense fluorescence upon binding to DNA. In the first series of experiments it was described effect on cellular ROS levels permeability.

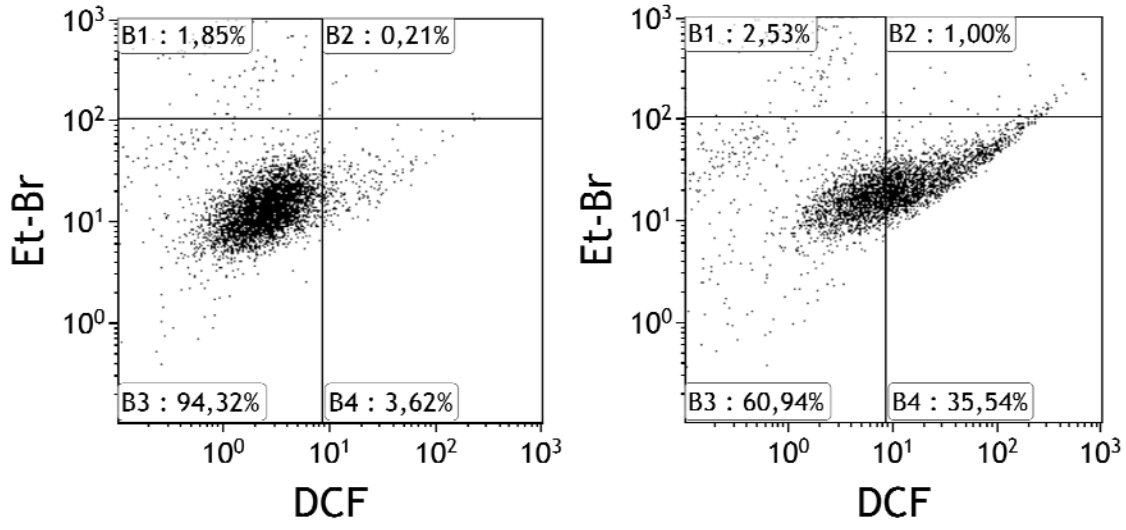

Fig. 3 - Comparison of cell death and intracellular ROS in cancer cell lines A549: 1-control; 2-10 μM (22*S*,23*S*)-28-homocastasterone.

As follows from Fig. 3, with the addition of synthetic (22*S*,23*S*)-28-homocastasterone there is a direct correlation between the intensity of the luminescence of cells caused by exposure to ethidium bromide (y-axis) and the level of reactive oxygen species (x-axis). This is due to the fact, that the increase in the level of ROS causes progressive disruption of the cell membrane permeability Et-Br, which is a sign of necrosis.

Thus, the tested compounds can be considered as potential antitumor agents. However, their mechanism of action is different: 24-epibrassinolide and 28-homocastasterone cause cell cycle arrest and apoptosis, which was previously shown on hor-

mone-dependent MCF-7 cell line.[3] The action of the synthetic brassinosteroids may be associated with an increased intracellular level of ROS damage to cell membranes, which can lead to cell necrosis.

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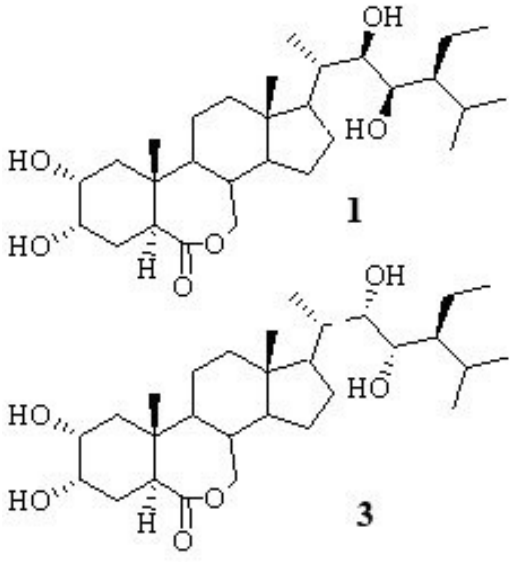
🕮 MOLECULAR MECHANISMS OF ANTIPROLIFERATIVE, CYTOTOXIC AND ANTICANCER EFFECTS OF BRASSINOSTEROIDS

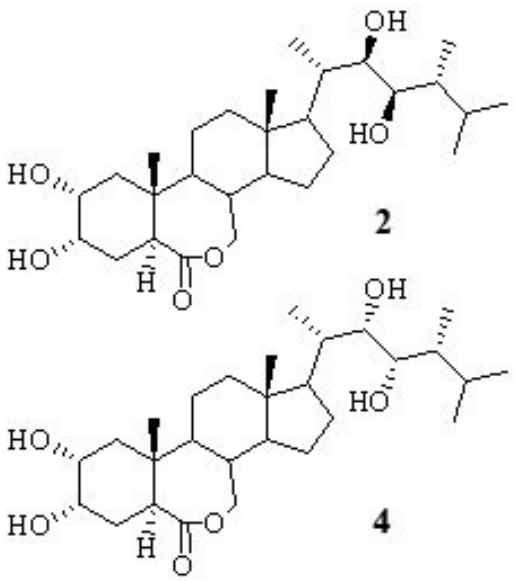
Aliaksei R. Sysa[1,2*], Pyotr Kisselev[1], and Olesya V. Panibrat[1]

[1] Institute of bioorganic chemistry of NAS of Belarus, Minsk, Belarus, [2] International Sakharov environmental institute of BSU, Minsk, Belarus
e-mail: aliaksei.sysa@iseu.by

Brassinosteroids (BS) are group of plant hormones similar to those of animals and humans in both structure and by function: they regulate expression of genes in plants, affect the metabolic processes, cell growth and differentiation. Recently there have been reports of a potential antiproliferative and anticancer activity of brassinosteroids with very low toxicity. The mechanism of action mostly is attributed to the blockade of the cell cycle by changing the expression level of cyclin-dependent protein kinases what leads to apoptosis.

It is proved the participating of monooxygenase systems in the initiation and development of many carcinogenic processes due to the activation of endogenous and exogenous compounds, e.g. estrogen steroids and polycyclic aromatic compounds. However, very little is known about the mechanism by which BS exert their cytotoxic effects. Knowledge of the influence of these compounds on the monooxygenase systems of animal cells is likely to contribute to the explanation of the observed effects.





In this paper, we tried to evaluate the effect of brassinosteroids on monooxygenases ability to activate procarcinogens in cell line A549 (lung cancer). Causal relationship between the development

of lung cancer and the change in the activity of cytochrome P-450 under the influence, in particular, components of tobacco smoke in no doubt. Thus, ten times the risk of lung cancer in smokers as compared to nonsmokers associated with the presence of PAHs in tobacco smoke.

To determine the contribution of monooxygenases to the carcinogenic activation we use the reaction of the epoxidation of dihydroxyderivatives of benzo[a]pyrene (B[a]P) (mainly 7,8-dihydroxydihydrobenzo[a]pyrene (B[a]P-7,8-diol)). This reaction ultimately leads to the formation of two stereoisomeric diolepoxydes, of which (+)-anti-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene ((+)-anti-BPDE) has absolute carcinogenic activity, while the other - no. Since formed diolepoxydes are unstable and easily hydrolyzed to the tetrol-derivatives, it is possible to determine the formation of an absolute carcinogen. We use four steroid compounds (28-homobrassinolide (**1**), 24-epibrasinolide (**2**), (22S,23S)-28-homobrassinolide (**3**) and (22S,23S)-24-epibrassinolide) (**4**) that differ in ring and side chain structure.

It was shown that in the A549 cell line the metabolism of B[a]P-7,8-diol is shifted toward the formation of derivatives which indicate the formation of the obligate carcinogen (+)-anti-BPDE. Cultivating of cancer cells in the presence of brassinosteroids changes the activity of monooxygenase system in general, as can be seen on the spending of the substrate, and changes the profile tetrol-derivatives what depends on the structure of BS.

We thank Dr. V. Khripach and Dr. V. Zhabinskii for samples of steroids used in this work, helpful discussion and comments.

🕮 BIOLOGICAL ACTIVITY OF NEW BRASSINOSTEROID ANALOGUES WITH PHENYL GROUP IN THE SIDE CHAIN

Jana Oklestkova*, Petra Kořínková, Miroslav Kvasnica, Jaromír Mikulík, Václav Bazgier, and Miroslav Strnad

Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany CAS & Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic.
e-mail: jana.oklestkova@upol.cz

Brassinosteroids (BRs) are polyhydroxylated steroidal plant hormones that play pivotal role in the regulation of various plant growth and development processes including cellular expansion and proliferation, vascular differentiation, fertility and senescence[1]. In addition to this, BRs have the ability to increase resistance to plants against various biotic and abiotic stresses[2]. These attributes make them good candidates for application in agriculture to improve growth and yield, but the major constraints to use BRs at large scale in the fields is their high cost. However, recent progress in the chemical synthesis of new biologically active brassinosteroids analogs leads to overcome this economically restriction.

We prepared a series of new brassinosteroid derivatives with *p*-substituted phenyl group in the side chain (Fig.1). All derivatives were successfully docking into the active site of BRI1 using AutoDock Vina and some compounds showed promisingly very strong interaction with BRI1 receptor. Plant biological activity was establish using the pea inhibition biotest and Arabidopsis root sensitivity assay and then was compared with natural brassinosteroids. Differences in the production of plant hormone ethylene were also observed in etiolated pea seedlings after treatment of brassinosteroid analogues.

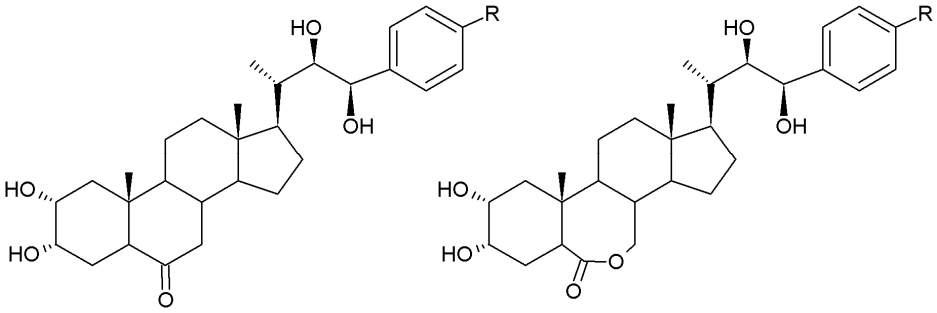


Fig.1: General structures of new brassinosteroids analogues.

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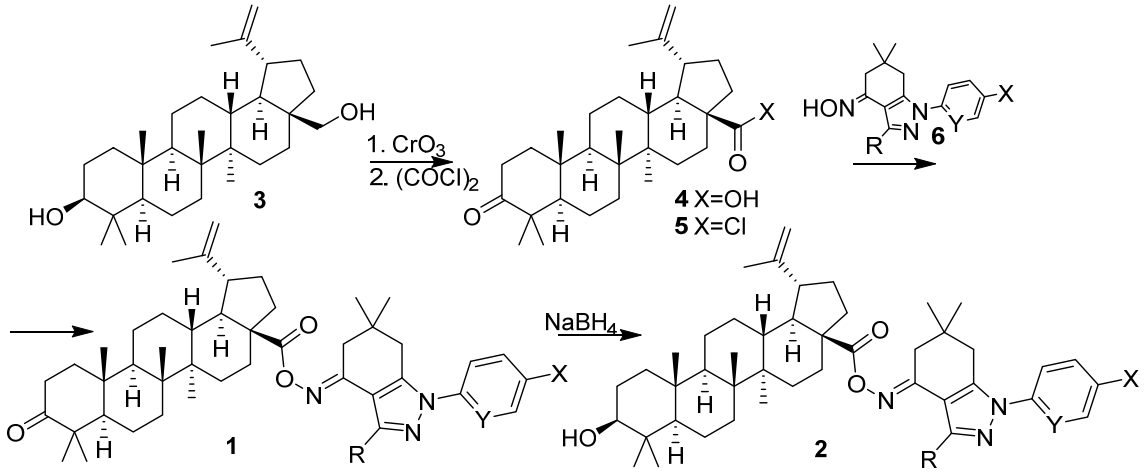
🕮 SYNTHESIS OF NOVEL LUPANE TRITERPENOID−INDAZOLONE HYBRIDS WITH OXIME ESTER LINKAGE

Tatyana S. Khlebnicova[1], Yuri A. Piven[1*], Fedor A. Lakhvich[1], Daina Zicāne[2], Inese Mieriņa[2], Uldis Peipiņš[2], and Māris Turks[2]

[1] Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Acad. Kuprevicha Str. 5/2, 220141 Minsk, Belarus, [2]Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, P. Valdena Str. 3, Riga, LV-1048, Latvia
e-mail: piven.ya@gmail.com

The hybridization of bioactive natural and unnatural compounds is one of the most promising for the design of new leading structures and the discovery of new and potent drugs in the field of medicinal chemistry. Betulin, betulonic and betulinic acids, naturally occurring pentacyclic lupane triterpenoids, are common secondary metabolites of plants, primarily from Betula species (Betulaceae), that exhibit a variety of biological activities[1]. An indazole skeleton is an attractive structural scaffold in medicinal chemistry. In particular, variously substituted tetrahydroindazolones were recently recognized as important anticancer drug candidates[2].



R = alkyl, perfluoroalkyl, 2-furyl, cyclopropyl; X = H, F; Y = H, N

An efficient protocol for the synthesis of novel lupane triterpenoid-indazolone hybrids **1,2** with oxime ester linkage has been developed from naturally accessible precursor betulin **3**. The synthetic pathway includes oxidation of betulin **3** to betulonic acid **4**, purification of the latter *via* its cyclohexylammonium salt, transformation of betulonic acid **4** into acid chloride **5**. For the first time a series of betulonic acid-indazolone hybrids **1** have been synthesized *via* an acylation of corresponding 6,7-dihydro-1*H*-indazol-4(5*H*)-one oximes[3,4] with betulonic acid chloride. Reduction of **1** with $NaBH_4$ in dry isopropanol occurred with full diastereoselectivity at C3 and led to the formation of betulinic acid-indazolone hybrids **2** in excellent yields.

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🕮 EFFICIENCY OF PLANT GROWTH REGULATOR «EPIN PLUS» (HOMOBRASSINOLIDE) ON CABBAGE

Feodor Popov[1] and Margarita Zavadskaya[2]

[1]Institute of plant protection of National academy of sciences of Belarus; [2]Institute of bioorganic chemistry of National academy of sciences of Belarus
e-mail: feodorpopov@yandex.ru

In modern vegetable growing, much attention is paid to plant growth regulators (PGR) because of their important role in the increasing of biological plant productivity. There are examples of successive application of PGR in production of cereal, vegetables, technical and other crops. Brassinosteroids and the preparations on their basis currently have scientific and practical interest.[1,2] Our scientific research provided creation of new methods and reglaments of application of preparation on the basis of brassinosteroids in vegetable growing with the aim of extention of their application sphere. In controlled conditions of laboratory experiment, effect of preparation Epin plus (0,25 g/l of homobrassinolide) was studied on sowing quality of cabbage seeds. It was estimated that optimal dose of the preparation for activation of seeds germination was 0,4 ml/kg. Energy of germination and laboratory germination capacity increased by 4 % in comparison with the control. A stimulating effect of phytoregulator on seedlings growth was shown. In variant with PGR the length of seedlings exceeded control value up to 31,6 %. Also decrease of affection of seedlings by complex of deseases was marked in experimental variants (1,1-1,2 times). In field conditions Epin Plus also increased germination capacity up to 88 % in comparison with 79 % in control, and have positive influence in sprout period. To the moment of formation of 5-7 real leaf, the plant height increased by 17,7 % relative to the control and assimilation surface of seedlings also increased by 1,4 times. The presowing treatment of seeds by preparation exerted sanitary influence resulted in decrease of seedlings affection by blackleg (8,2–9,3 % in experiment, 14,7 % in control) that favored to increase the yield of healthy seedlings from area unity by 12,8 %.

In the vegetation period Epin Plus was applied to cabbage plants in the phase of head setting. As a result of the treatment, the increase of head weight and its diameter by 20,7 % and the yield increase by 44,0 c/ha was observed.

In conclusion, based on the results of our study, the preparation "Epin plus" has been recommended for registration and for the use in cultivation of cabbage.

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🕮 ANTICANCER ACTIVITIES OF BRASSINOSTEROIDS AND THEIR DERIVATIVES

Lucie Rárová[1], Jana Oklestkova[2], Jana Steigerová[3], and Miroslav Strnad[2]

[1] Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR & Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic, [2] Laboratory of Growth Regulators, Institute of Experimental Botany ASCR & Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic, [3] Laboratory of Molecular Pathology, Department of Clinical and Molecular Pathology, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech Republic
e-mail: lucie.rarova@upol.cz

Polyhydroxylated sterol derivatives with close structural similarity to animal and insect steroid hormones, brassinosteroids (BRs), are plant growth regulators representing a group of newly-discovered agents with relatively wide-ranging effects in plants. The study of plant-derived compounds and their analogues with effect at the molecular level has become an important approach in the selection of new agents with antitumour activity in humans. Molecular and cellular effects of natural BRs and their derivatives were examined in different human cancer cell lines and in primary endothelial cells *in vitro*. BRs and analogues caused growth inhibition, cell cycle arrest and initiation of apoptosis in many different cancer cell lines and also inhibition of angiogenesis *in vitro* in human endothelial cells. These findings indicate a potential use of BRs in the prevention of metastasis development. Investigation of the mechanisms of action of BRs and derivatives in human cancer and endothelial cells using cellular and molecular techniques indicated the possible involvement of steroid receptors in BR action. However, BRs were shown not to bind directly to steroid receptors which demonstrate that BRs act via steroid receptor-independent pathway(s). Our results suggest that tested BRs and their analogues are promising leads for the development of a new generation of potential anticancer drugs.

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🕮 STRUCTURE OF 15,16-EPOXY-TRANS-CLEROD-3,13(16),14-TRIENE,19,6β-OLIDE FROM *PULICARIA SALVIIFOLIA* BUNGE

Parasat E. Sakenova[1], Yuriy V. Gatilov[2], Gayane A. Atazhanova[1], and Sergazy M. Adekenov[1]

[1]International Research and Production Holding "Phytochemistry", Karaganda Republic of Kazakhstan; [2]N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry of Siberian Branch of Russian Academy of Sciences, Novosibirsk
e-mail: phyto_pio@mail.ru; gatilov@nioch.nsc.ru

Pulicaria — a genus of grassy plants of Asteraceae family which includes 63 species. Species of genus are occured in Eurasia and Africa, and mainly concentrated in the Mediterranean. On the territory of

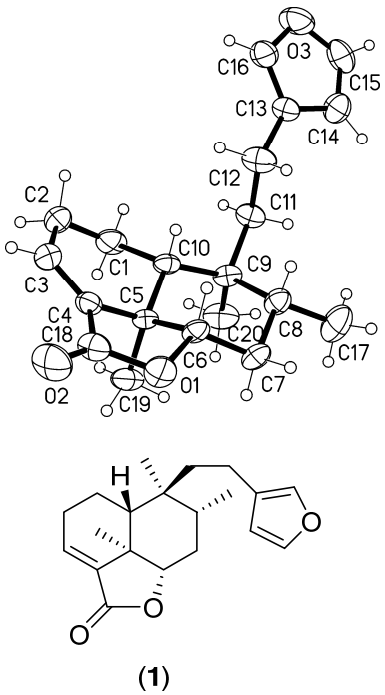
the CIS plants of *Pulicaria* genus grow in the European part, Central Asia, Western Siberia, the Caucasus and the Far East, and there are only 5 species, which 4 species of them grow on the territory of Kazakhstan.[1]

Earlier[2] from elevated part of *Pulicaria salviifolia* Bunge. extraction by chloroform and chromotographic division of the sum of substances on column with silica gel was allocated diterpenic lactone salvin according to IR - mass, Nuclear Magnetic Resonances ranges the probable stereochemistry of molecule is offered.

Elevated part of *Pulicaria salviifolia* Bunge., collected on the territory of the Southern Kazakhstan region, in the neighborhood of the village Achisay we extracted supercritical carbon dioxide with the subsequent division by method of the centrifugal chromatography of distribution (CCD) on the FCPC-A200 installation. At re-chromatography of lactone fraction on silica gel with the KSK brand (the ratio the sum carrier 1:15), colourless crystal substance of structure $C_{20}H_{26}O_3$ with m.t. 129-132ºC (petroleum air ethyl acetate) is allocated (1).

On the base of spectral data (IR -, ^1H and ^{13}C of nuclear magnetic resonance) and physical and chemical constant substance is identified as salvin (1), crystal structure of molecule which is studied for the first time by method of the X-ray diffraction analysis by us.

According to the results of X-ray diffraction experiment is revealed that the lactone ring has the β-configuration relative to the carbon skeleton, a methyl group at C-5 is β-oriented (Fig. 1). The methyl group at C-2 is also located in the β-position. Conformation hexene cycle is close to the envelope with the release of the atom C-10 to 0,713, and 0.695 Å, corresponding for the two molecules. The hexane cycle in conformation of slightly distorted chair.



(**1**)

Figure 1. Spatial structure of molecule (**1**)

Lactone cycle has the shape of half-chair with the release of the plane atoms C5,C6 to +0,334, -0,348 and +0.320, -0.359 Å. In the Cambridge base of Cambridge Structural Database.[3] There are data of structure the closest analogue cis-clerodanfuranelactone,[4] which is different from the salvin by atom configuration at C-5. In the crystal of the molecule Salvin with weak hydrogen bonds C16-H... O2 (distance H... O 2.34, 2.37 Å, the angle C-H ... O 165, 166º) are connected to the tape along the axes of a and b.

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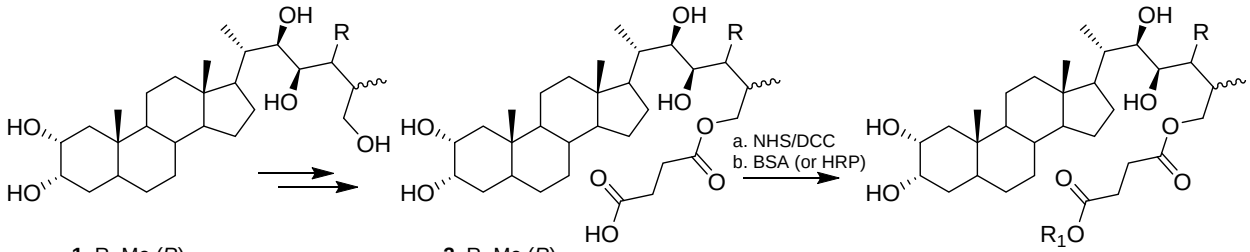
🕮 TEST-SYSTEMS FOR IMMUNOASSAY OF 6-DEOXOBRASSINOSTEROIDS

Alina L. Sauchuk*, Raisa P. Litvinovskaya*, Andrey G. Pradko, and Tatyana V. Mirantsova****

**Institute of Bioorganic Chemistry NAS of Belarus, 220141 Minsk, Kuprevich Str., 2/5, Belarus;*
***Hemma-Test Ltd., 220029 Minsk, Masherova St., 11, Belarus*
e-mail: alina_kyrtikova@mail.ru

A special group of brassinosteroid plant hormones are 6-deoxobrassinosteroids, which are biosynthetic precursors of the corresponding BS ketones, such as castasterone, 24-epicastasterone, 28-homocastasterone and lactones (brassinolide, 24-epibrassinolide, 28-homobrassinolide at all.). It has been found that 6-deoxocastasterone and 6-deoxocathasterone having a significant biological activity are the most common in plant tissues along with brassinolide.

Here we report our results on design of test-systems for 6-deoxobrassinosteroids enzyme immunoassay (ELISA) whereby their quantitative analysis in plant tissues can be done.



1. R=Me (*R*)
2. R=H

3. R=Me (*R*)
4. R=H

5. R=Me (*R*), R_1=BSA
6. R=H, R_1=BSA
7. R=Me (*R*), R_1=HRP
8. R=H, R_1=HRP

Haptens 3 and 4 with spacer at position C-26 have been prepared from 26-hydroxy-6-deoxo-24-epicastasterone 1 and 26-hydroxy-6-deoxo-28-norcastasterone 2, respectively. They reacted with bovine serum albumin (BSA) to give the conjugates 5 and 6 used as immunogens for producing the antisera specific for 6-deoxo-24-epicastasterone and 6-deoxo-28-norcastasterone, respectively. Horseradish peroxidase (HRP) was conjugated with haptens **3** and **4**, obtained conjugates **7** and **8** were used as labeled antigens. Highly specific antibodies were obtained by immunization of two groups of rabbits with conjugates 5 and 6. So, obtained antibodies and labeled antigens were the basis for design of test-systems for ELISA of 6-deoxo-24-epicastasterone (ELISA-1) and 6-deoxo-28-norcastasterone (ELISA-2).

The obtained antibodies have 100% specificity for the determination of 6-deoxo-24-epicastasterone and 6-deoxo-28-norcastasterone. Other 6-deoxo-BS have showed a lower cross- reactivity (~ 13-38%) in both test-systems. Only a small cross reactivity (0.01-4.07%) was observed for 6-keto- and B-lactone-BS. There was no cross-reactivity detected with non-BS steroids.

The developed test-systems are very sensitive. The limit of detection was found to be 0.01 nmol/L for both cases. An analytical working range is 0.01-3 nmol/L (part of the curve between 40% and 90%). Accuracy studies, parallelism and imprecision data were determined and all were found to be satisfactory. The main characteristics of the immunogenic test-systems are shown in Table. Some chemical and spectral properties of new synthesized compounds and cross-reactivity of various steroids in the ELISA-systems will be presented.

Table - Main characteristics of the immunogenic test-systems

Parameter	Test-system	
	ELISA-1	ELISA-2
Accuracy	96.4 ± 7.6%	92.8 ± 9.6 %
Parallelism	10.1-14.8%	10.5-16.7%
Imprecision	8.3-14.3%	9.5-15.8%

🕮 SYNTHESIS AND STRESS-PROTECTIVE ACTION OF BRASSINOSTEROID SALICYLATES

Aleh Savachka[1]*, Raisa P. Litvinovskaya[1], Andrei Vayner[2], Halina Zhylitskaya[1], and Yurii Kolupaev[2]

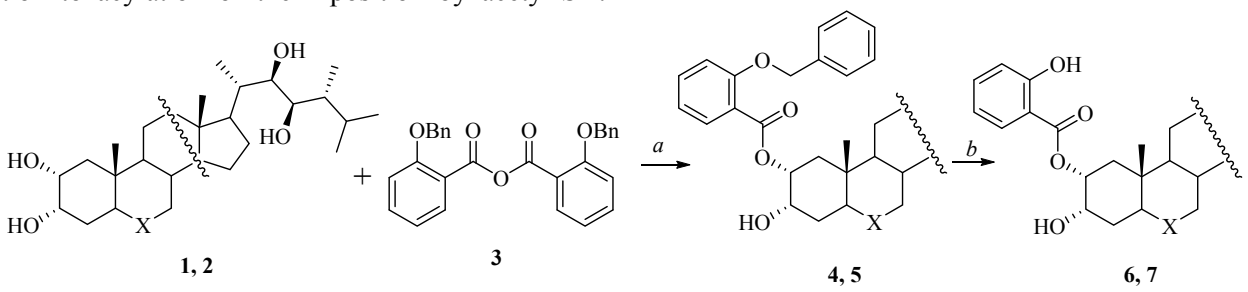
[1] Institute of Bioorganic Chemistry NAS of Belarus, Minsk, Belarus, [2] V. V. Dokuchaev Kharkov National Agrarian University, Kharkiv, Ukraine
e-mail: oleg.brsv@mail.ru

Brassinosteroids (BS) are well known as the phytohormones with a high plant-growth-promoting and stress-protecting activity[1]. Literature data on their functional interaction with other phytohormones and plant bioregulators prompted us to synthesize BS-conjugates with salicylic acid (SA) and to assess their stress-protective activity.

Attempts to prepare BS-salicylates using SA anhydride or chloride showed that the acylation was non-specific and formed a complicated product mixture. A study of the reaction of BS with an excess of acetyl-SA anhydride, which was prepared in situ from the acid through the action of dicyclohexylcarbodiimide (DCC) in dioxane, showed that 3,22,23-triacetoxy-2-(2'-acetoxy-benzyloxy)-derivatives were formed, i.e., acetylation of the hydroxyls at C-3, C-22, and C-23 occurred in addition to acylation of the 2-position by acetyl-SA. All esters were saponified under the hydrolysis conditions so that monoesters of acetyl-SA could not be obtained.

2-O-Benzylsalicylic acid, which was prepared from SA by refluxing in acetone with an excess of benzyl bromide in the presence of K_2CO_3 followed by saponification of the resulting ester, was used as a reagent. 2-Acyl-derivatives **4** and **5** were synthesized by reaction of 24-epibrassinolide (**1**, 24-EBl) and 24-epicastasterone (**2**, 24-EBk), respectively, with 2-O-benzylsalicylic acid anhydride (**3**), which was prepared in situ from the acid. The reaction occurred at room temperature with a slight excess of anhydride **3**.

The benzyl protection in **4** and **5** was removed by hydrogenolysis in MeOH over a Pd catalyst. This produced target products **6** and **7**.



1, 2 **3** **4, 5** **6, 7**

1, **4**, **6**: X = CO-O; **2**, **5**, **7**: X = CO

a. Dioxane, DMAP, 20°C, 24 h; *b*. H_2/Pd, MeOH, 20°C, 1 h

The physiological activity of SA-modified BS was assessed by comparing the effects of 24-EBl and 24-EBk and their SA-conjugates on the heat- and salt-stress resistance of young millet plants Panicum miliaceum L. We demonstrated earlier that the experimental plants were sensitive to the action of BS, which at concentrations of 10^{-8}–10^{-6} M increased the heat resistance of the sprouts. The effect of BS was greatest at a concentration of 10^{-7} M (Table 1). The positive effects of the conjugates of 24-EBl and 24-EBk with SA at concentrations of 10^{-8} and 10^{-7} M were greater than those of 24-EBl and 24-EBk at the corresponding concentrations. The positive effects of BS and their conjugates decreased at a concentration of 10^{-6} M although the differences between the variants disappeared. Mixtures of BS and SA at concentrations of 10^{-8} and 10^{-7} M did not enhance the heat resistance of the sprouts whereas higher concentrations decreased the survival of sprouts after heating.

Table 1. Effect of physiologically active compounds on survival of millet sprouts after heat stress (10 min at 47°C).

Variant	Concentration, M		
	10^{-8}	10^{-7}	10^{-6}
	Survival, %		
Control		45.2 ± 1.4	
1	50.4 ± 1.3	59.2 ± 1.2	54.1 ± 1.8
2	51.5 ± 1.2	57.6 ± 1.5	54.4 ± 1.4
SA	50.2 ± 1.5	54.7 ± 1.3	49.3 ± 1.2
1 + SA mixture	47.9 ± 1.7	43.2 ± 1.2	37.4 ± 1.5
2 + SA mixture	48.0 ± 1.4	44.1 ± 1.7	34.4 ± 1.8
6	58.8 ± 1.6	66.9 ± 1.4	57.4 ± 1.6
7	57.4 ± 1.4	65.3 ± 1.1	53.8 ± 1.4

The effects of BS and their SA conjugates on the stress-resistance of young millet plants to salt were compared in the next series of experiments. Treatment of millet plants with BS modified by SA had a more substantial protective effect than the corresponding one initiated by non-modified BS and SA (Fig. 1).

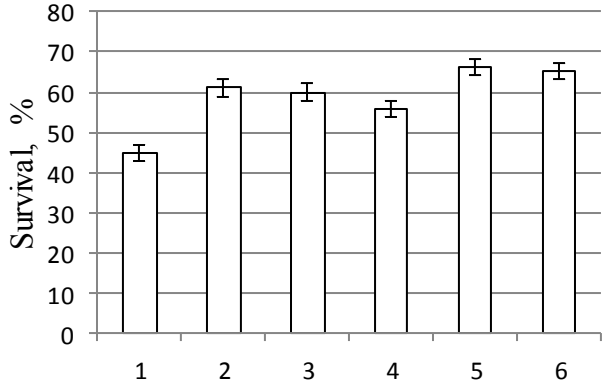


Fig. 1. Effects of **1**, **2**, SA, and conjugates **6** and **7** on salt-stress resistance of millet sprouts treated with NaCl solution (500 mM) for 7 h: control (1), **1** (0.1 μM) (2), **2** (0.1 μM) (3), SA (0.1 μM) (4), **6** (0.1 μM) (5), and **7** (0.1 μM) (6).

Thus, the effects of conjugates of BS with SA (BS-salicylates) differed substantially from those of a mixture of BS and SA and their separate application. These differences may have been related to gradual release of free BS and SA from the conjugates whereas the two phytohormones were antagonistic if administered simultaneously. The combination of stress phytohormones BS and SA as chemical conjugates may have initiated a broader spectrum of protective reactions than BS and SA separately.

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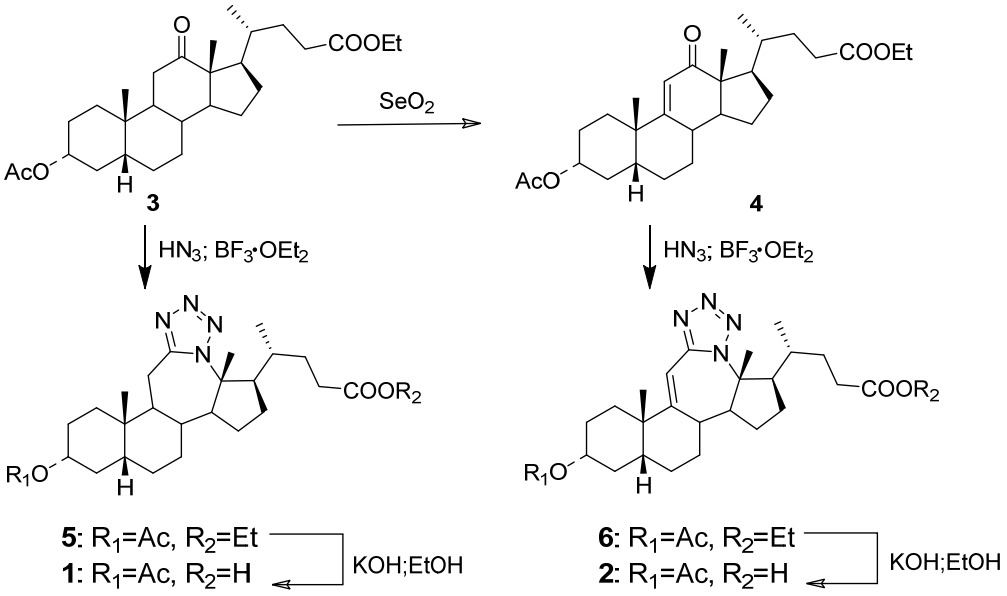
🕮 SYNTHESIS OF SOME NEW BILE ACID TETRAZOLE DERIVATIVES FROM DEOXYCHOLIC ACID

Dušan Škorić[1], Marija Sakač[1], and Janoš Čanadi[1]

[1] Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, R. Serbia
e-mail: dusan.skoric@dh.uns.ac.rs

Bile acids and their derivatives are a popular topic for research due to their various applications in medicine and pharmacy especially as new drugs and drug transport promoters[1]. Important field of research in bile acid chemistry is synthesis of new derivatives with improved biological and pharmacological properties (better bioavailability, decreased membrane toxicity etc.). Having in mind widespread use of tetrazole moiety in the synthesis of pharmaceutically applicable substances and unique physico-chemical and pharmacological properties that tetrazole can provide, we became interested in the synthesis of tetrazole derivatives of bile acids. In this work we present synthesis of two new bile acid derivatives bearing C-ring fused tetrazole: 12a-aza-3α-hydroxy-12a-homo-tetrazolo[5'1' : 12,12a]-5β-holan-24-oic acid (**1)** and 12a-aza-3α-hydroxy-12a-homo-tetrazolo[5'1': 12,12a]-5β-hol-9(11)-en-24-oic acid (**2)**.

The key intermediate for synthesis of these tetrazole derivatives was ethyl 3α-acetoxy-12-oxo-5β-holanoate (**3**) which was prepared in high yield from deoxycholic acid (DCA) through selective protection[2] and subsequent oxidation of the protected DCA. Selenium dioxide dehydrogenation of compound **3** afforded enone **4** in 72% yield. Ketone **3** and enone **4** were reacted with excess of hydrazoic acid in the presence of boron trifluoride diethyl etherate as Lewis acid catalyst[3] to form corresponding tetrazoles **5** and **6** in 74% and 62% yield, respectively. Considerably lesser reactivity of enone compared to ketone **3** was noticed. Finally total deprotection with ethanolic potassium hydroxide and acidification gave the free acids **1** and **2**. Structures of all newly synthesized compounds were extensively investigated by NMR spectroscopy and other spectroscopic techniques.



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🕮 DEVELOPMENT OF LC-MS/MS METHOD FOR SIMULTANEOUS DETERMINATION OF ESTROGENS AND SELECTED ENDOCRINE DISRUPTORS IN HUMAN PLASMA

Lucie Kolatorova Sosvorova*, Tereza Chlupacova, Jana Vitku, Marie Bičíková, Richard Hampl, and Luboslav Starka

Institute of Endocrinology, Department of Steroids and Proteofactors, Prague, Czech Republic
e-mail: lsosvorova@endo.cz

Introduction: The current population is exposed to number of chemicals that can affect endocrine system. These substances are called endocrine disruptors (EDs) and among them bisphenol A (BPA) is one of the most widely studied. Its mechanism of action through binding on different types of estrogen receptor and interfering with endogenous estrogen action is known. In several countries, the use of BPA occurring predominately in plastics is now regulated or prohibited. However, BPA is often replaced by other bisphenols as bisphenol S (BPS), bisphenol F (BPF) or bisphenol AF (BPAF). The estrogenic potential of mentioned bisphenols is similar and in several cases even stronger than BPA. Monitoring and evaluating the levels and effects of BPA alternatives is still in the beginning and their use in not regulated.

Another widespread EDs are parabens, widely used antimicrobial agents predominately in cosmetics and pharmaceutic products. After finding their estrogenic activity, their usage was regulated by European Union. The number of scientific papers documenting the endocrine system disruption by parabens rapidly increased, however the information of direct effect of selected parabens to the human endocrine system are still missing.

The aim of the study was to develop and evaluate the liquid chromatography – tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of estradiol (E2), estrone (E1), estriol (E3), BPA, BPS, BPF, BPAF, methylparaben (MP), ethylparaben (EP) and propylparaben (PP) in human plasma.

Methods: Different extraction agents were tried out (diethyl ether, methyl tert-butyl ether). Furthermore, derivatization step at phenol moiety with dansyl chloride was carried out. Also the chromatographic conditions (colon, flow, temperature) were tested. Subsequently the parameters of MS/MS detector for individual analytes were optimized in positive ESI mode.

Results: Due to phenolic hydroxyl group occurring in all compounds of interest we were able to derivatize and detect all analytes in one run. After optimization of all parameters we can determine above mentioned analytes from 500µl of plasma. The method enabled determination of plasma BPA, BPS, BPF, BPAF, MP, EP, PP, E2, E1 and E3 with the interassay and intraassay coefficients of variation within 15% and recovery within 85 – 115%. Deuterated internal standards were employed for the correction of losses during sample preparation.

Novel aspect: This is the first method allowing determination of selected bisphenols, parabens and estrogens in one run.

Acknowledgement: This study was supported by MH CZ – DRO (Institute of Endocrinology – EU, 00023761) from the Ministry of Health of the Czech Republic.

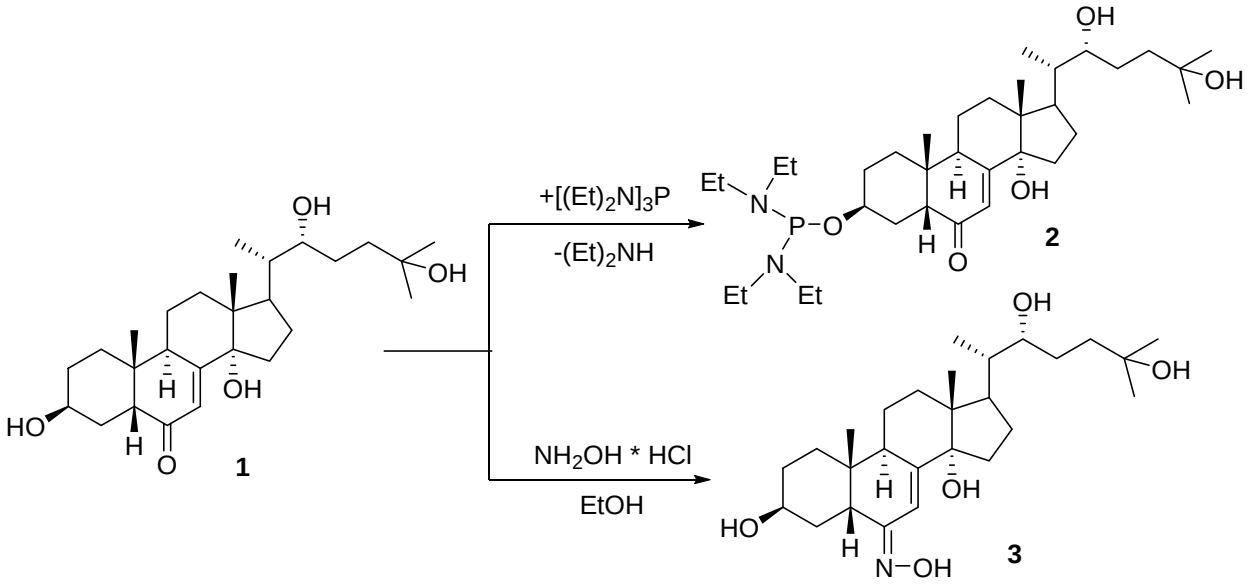
🕮 REGIOSELECTIVE SYNTHESIS OF NEW PHOSPHORUS- AND NITROGEN- 2-DEOXYECDYSONE-BASED DERIVATIVES

Bakhtiyar S. Temirgaziyev[1], Lyazzat K. Salkeyeva[2], Gulnara S. Agitayeva[1], Aizhan M. Kozhanova[1], Borash I. Tuleuov[1], and Sergazy M. Adekenov[1]

[1]JSC International research and production holding "Phytochemistry", 4 M. Gazaliyev St., Karaganda, 100009, phone/fax: (87212)433127; [2]Karaganda State University named after E.A. Buketov, 28 Universitetskaya St., Karaganda, 100028, phone/fax: (87212) 770385
e-mail: phyto_pio@mail.ru; Lsalkeeeva@mail.ru

We have first recovered 2-deoxyecdysone (3β,14α,22R,25-tetrahydroxy-5β(H)-cholest-7-en-6-on) from the aerial parts of *Silene cretaceae* Fisch of *Caryophyllaceae* Juss. family, and from the aerial parts of *Silene fruticulosa* (Pall.) Schischk. in quite a high concentration – 0.45 g/kg[1].

Therefore, the goal of this paper is regioselective chemical modification of 2-deoxy ecdysone – the most important technically available synthon, also recovered by us from species-superproducer – *Silene wolgensis* (Hornem) Bess. of the aforesaid family.



Regioselective reaction of 2-deoxyecdysone (**1**) with hexaethyltriamidophosphite (HETAP) was performed in the medium of absolute benzene at 60°C within 48 hours, while the reaction of ketoximation of compound (**1**) with hydrochloric hydroxylamine was performed in absolute benzene at 60^0C within 72 hours. Thus, the results of reaction between 2-deoxy ecdysone (**1**) with HETAP and NH_2OH•HCl lead to the formation of target phosphorus and nitrogen derivative (**2**) and (**3**) obtained with 92 and 74% yield respectively.

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🕮 SPECTRAL AND MICROSCOPIC STUDY OF SELF-ASSEMBLY OF NOVEL CATIONIC SPERMINE AMIDES OF BETULINIC ACID

Uladzimir Bildziukevich[1,2], Eva Kaletová[1], David Šaman[3], Elina Sievänen[4], Erkki Kolehmainen[4], Miroslav Šlouf[5], and Zdeněk Wimmer[1,2*]

[1] Institute of Experimental Botany AS CR, Isotope Laboratory, Vídeňská 1083, 14220 Prague 4, Czech Republic, [2] University of Chemistry and Technology in Prague, Department of Chemistry of Natural Compounds, Technická 5, 16628 Prague 6, Czech Republic, [3] Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo náměstí 2, 16610 Prague 6, Czech Republic, [4] University of Jyväskylä, Department of Chemistry, 40014 Jyväskylä, Finland, [5] Institute of Macromolecular Chemistry AS CR, Heyrovský sq. 2, 16206 Prague 6, Czech Republic
e-mail: wimmer@biomed.cas.cz; Zdenek.Wimmer@vscht.cz

Supramolecular characteristics of two spermine amides of betulinic acid (**1** and **2**) were studied by measuring and evaluating their UV-VIS-NIR spectra in aqueous acetonitrile and DOSY-NMR spectra in tetradeuteromethanol, accompanied by atomic force microscopy (AFM) images (Figure 1), scanning electron microscopy (SEM) micrographs (Figure 2), and transmission electron microscopy (TEM) micrographs (Figure 2)[1]. Fibrous supramolecular self-assembly of **1** and **2** was observed by AFM images, as well as by the SEM and TEM micrographs. Bathochromic shifts of the absorbance maximum at 870 nm to 1015–970 nm in the UV-VIS-NIR spectra were observed with increasing water content in the acetonitrile/water systems, indicating formation of fibrous *J*-type aggregates. Variable temperature DOSY-NMR spectral measurement showed non-linear dependence that also suggests self-assembly behavior of the studied systems (Figure 3).

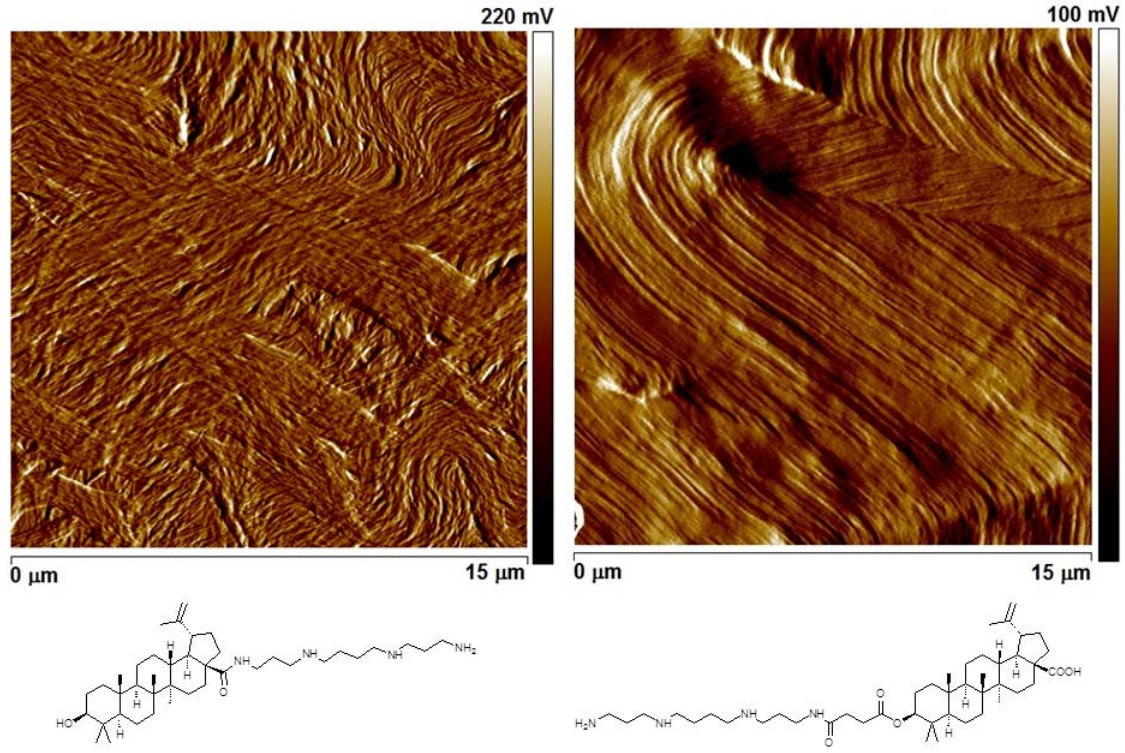

Figure 1: AFM images and structures of the compounds **1** (left) and **2** (right).

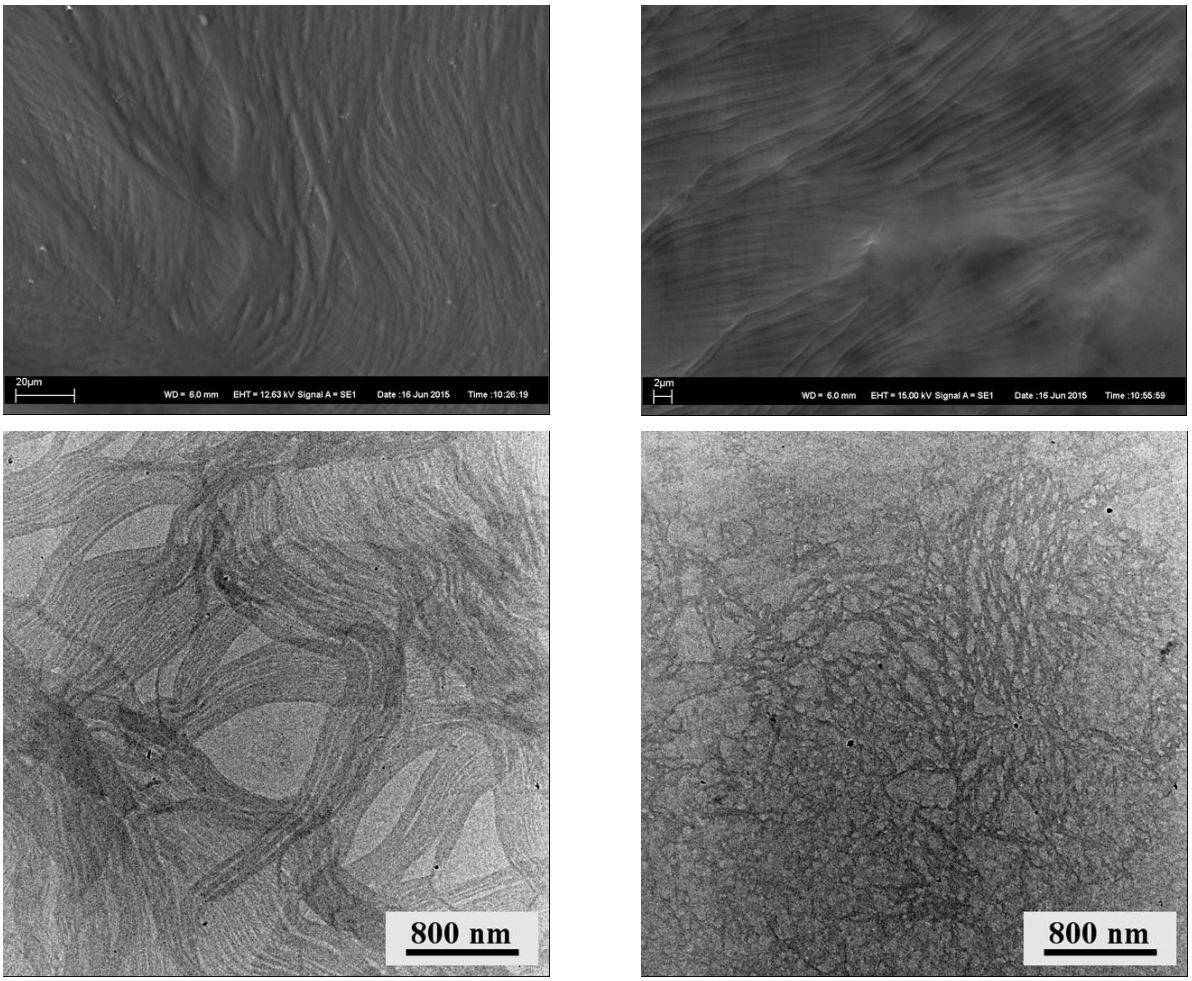

Figure 2: SEM micrographs of **1** (top left) and **2** (top right) and TEM micrographs of **1** (bottom left) and **2** (bottom right).

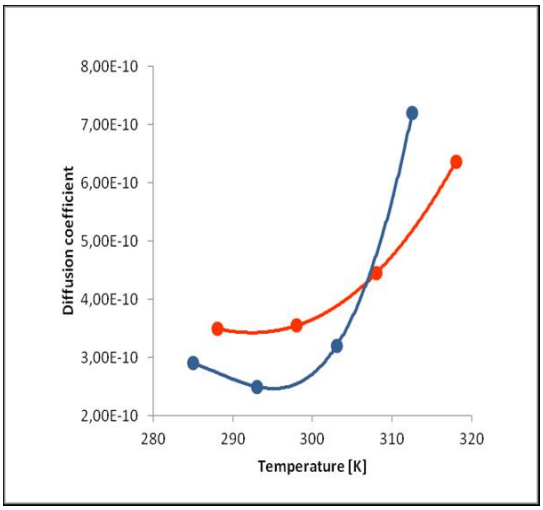

Figure 3: Temperature dependence of the diffusion coefficient measured by the variable temperature DOSY-NMR technique of compounds **1** (red) and **2** (blue).

Chiral supramolecular structures were formed by self-assembling due to the chirality of the monomeric molecules. Application of aqueous media during self-assembly procedures is an important factor in the development of targeted drug delivery systems.

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🕮 THE STUDIES ON THE REACTION OF 16-DEHYDROPREGNENOLONE ACETATE WITH 2-AMINOBENZIMIDAZOLE

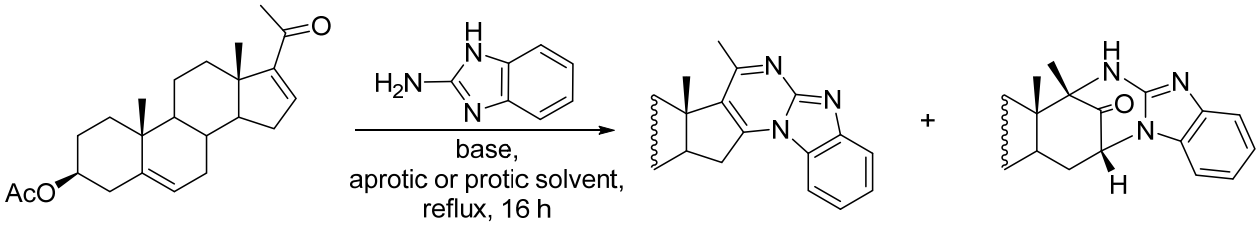
Agnieszka Wojtkielewicz*, Paulina Uścinowicz, Leszek Siergiejczyk, Urszula Kiełczewska, Artur Ratkiewicz, and Jacek W. Morzycki

Institute of Chemistry, University of Białystok, K. Ciołkowskiego 1K, 15-245 Białystok, Poland
e-mail: jeremy@uwb.edu.pl

Synthesis of benzimidazo[1,2-a]pyrimidines has gained considerable interest because of the pronounced biological activity of these compounds. They were reported to have antibacterial, anticancer, sedative, and antiarrhythmic.[1] Some of them (fasiplon, taniplon, divaplon) have found application as anxiolytic drugs.[2] However only a few steroid fused pyrimido[1,2-a]benzimidazoles have been described so far.[3] Since nowadays there is a tendency to combine two pharmocophores in one hybrid molecules, which potentially is more effective than its individual components, we attempted to connect steroid skeleton with benzimidazo[1,2-a]pyrimidine.

To synthesize the steroidal pyrimidobenzimidazole derivative we have carried out the condensation of 16-dehydropregnenolone acetate with 2-aminobenzimidazole under various reaction conditions (Scheme 1). As 2-aminobenzimidazole possesses two nucleophilic nitrogen atoms (NH_2 and N3) the condensation may lead to two isomeric pyrimidines. However, the aromatic product was formed regioselectively, by conjugated addition of N3 of benzimidazole to C16 of steroid, followed by cyclization, autoxidation, and aromatization. Unexpectedly, the major product was accompanied by the D-homo ketone produced by competitive α-ketol type rearrangement of the hydroperoxide intermediate. The tentative mechanism is postulated.

The elucidation of the product structures was carried out by NMR, IR, MS and X-ray methods as well as chemical evidences.



Scheme 1. The reaction between 16-dehydropregnenolone acetate and 2-aminobenzimidazole

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🕮 SYNTHESIS OF NEW TRILOBOLIDE DERIVATIVES

Tomáš Zimmermann[1] and Pavel Drašar[1*]

University of Chemistry and Technology, Technická 5, 166 28 Praha 6 Dejvice
e-mail: tomaszimmermann6@gmail.com.

Trilobolide (Tb) is sesquiterpene lactone isolated from *Laser trilobum* (L.) Borkh. Tb and other compounds belonging to group called thapsigargines are known to inhibit activity of sarco/endoplazmic reticulum Ca^{2+} ATPase (SERCA).[1] Inhibition of SERCA and rise of cytosolic concentration of Ca^{2+} may lead to apoptosis. Tb has also interesting immunostimulatory activity, such as interferon-γ and NO production increase.[2]

In our work several types of derivatives were prepared. One of them is 7-α-alkylestradiol which might possibly combine antiestrogenic and apoptotic activities. The other derivatives are supposed to better our knowledge about the binding of Tb within SERCA pump. Nine new compounds designed *„in silico"* were synthesised. *In vitro* testing will be carried out to determine biological activity of these derivatives.

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