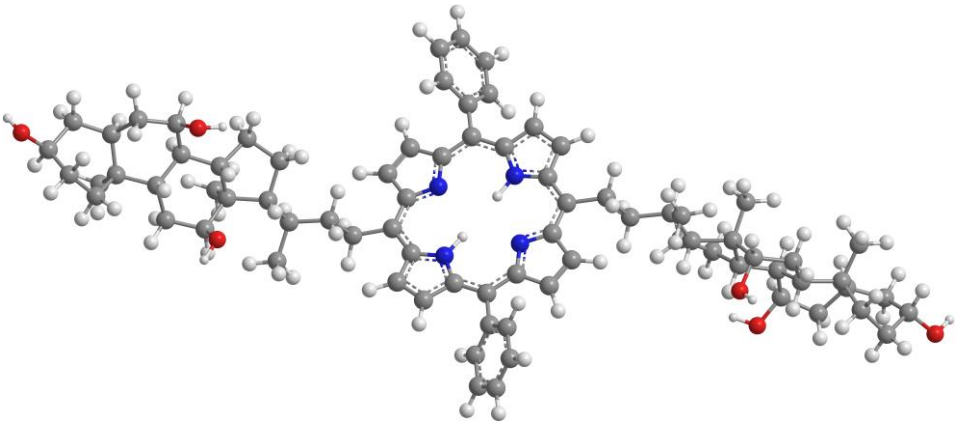

22nd Conference on Isoprenoids

Prague, Czech Republic, September 7 — 10, 2014

Abstract Book
edited by
Radmila Řápková, Michal Jurášek, Pavel Drašar

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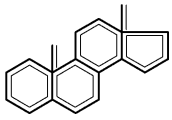
Resurrection of Isoprenoids



The conference was originated by prof. Marian Kocór from the Warsaw Institute of Organic Chemistry of the Polish Academy of Sciences (ICHO PAN) and in the beginning it was held four times in Poland (Warszaw 1966; Szklarska Poręba 1967; Karpacz 1969; Jadwisin 1971) as a conference purely on steroids. On the agreement of prof. Kocór with then the director of the Prague Institute of Organic Chemistry and Biochemistry Czechoslovak Academy of Sciences (IOCB) Prof. Vlastimil Herout and then the head of the IOCB department of steroid chemistry dr. Václav Černý the conference was transferred to Bohemia, to Liblice castle (north of Prague, CZ) in 1973 and became biannual Czechoslovak-Polish meeting on Isoprenoids. To complete the Czecho-Slovak side we shall name also prof. Jozef Tomko (Pharm. Inst. Komenius University Bratislava, SK) as one of the "founding fathers".

Then, the conference went on: Toruń, PL, 1975; Tatranská Lomnica, CZ, 1977; Toruń, PL, 1979; Praha, CZ, Sept. 1981; Třeboň, CZ, Oct. 16-21, 1983; Jachranka, PL, Sept. 16-21, 1985; Pec pod Sněžkou, CZ, Oct. 4-11, 1987; Poznaň, PL, Sept. 24-29, 1989; Tábor, CZ, Sept. 15-21, 1991; Zakopane, PL, 1993; Praha, CZ, Sept. 17-23, 1995; Krakov, PL, Sept. 21-26, 1997; Prachatice, CZ, Sept. 10-16, 1999; Gdańsk, PL, Sept. 8-14, 2001; Liberec, CZ, Aug. 12-18, 2003; Białowieża, PL, Sept. 23-29, 2005, where was held the 21st conference, to be followed by the 22nd in Praha, CZ, in Sept. 7-10, 2014.

Long list of shining names made this conference on one side the unique source of the information from the discipline and also the working transmembrane channel through the Iron Curtain. To name some of the great Isoprenoids names: Abubakirov N. K., Adam G., Akhrem A. A., Ananchenko S., Appendino G., ApSimon J., Arigoni D., Atta-Ur-Rahman, Back T. G., Baeckstrom P., Barton D. H. R., Birch A. J., Boeckmann R., Bohlmann F., Boland C. W., Brosa C., Buděšínský M., Canonica L., Caspi E., Černý V., Coates R. M., Connolly J. D., Covey D. F., Daniewski A. R., Daniewski W. M., Dauben W. G., De Clerq P., de Groot A., DeLuca H. F., Dreiding A., Fajkoš J., Feringa Ben F., Franke W., Frelek J., Harmatha J., Herout V., Holub M., Hudlicky T., Ito Sho, Jommi G., Kalvoda J., Kamano Y., Kamernitzky A. V., Kasal A., Khripach V., Kitahara T., Kocienski P., Kočovský P., Kohout L., Kraus W., Kreiser W., Meinwald J., Mori Kenji, Morzycki J., Nakamura E., Nakanishi K., Nicolaou K. C., Norin T., Noyori R., Okamura W. H., Oppolzer W., Ourisson G., Piozzi F., Prestwich G. D., Schonecker B., Schreiber K., Schubert K., Senatore F., Snatzke G., Šorm F., Sukh Dev, Tietze L. F., Tomko J., Torgov I. V., Trost B. M., Vidari G., Vincze I., Vita-Finzi P., Welzel P., Wenkert E., Wessjohan L. A. Wicha J., Wiechert R., Wiesner K., Yokota T., Zbiral E., Zeelen F. J., Zhabinsky V., to name just few.

After Białowieża meeting the crew of organisers seemingly lost the driving force and even some people considered isoprenoid chemistry as boring and finished, where nothing exciting and important cannot be found any more, and went with the conference series to the dead end. However, since 2005 there have been achieved many exciting findings in the field, especially on the borderline between disciplines and in newer fields as e.g. the supramolecular chemistry. I remember i.a. that betulin and its derivatives were considered as typical compounds interesting just for their structure and easy isolation, and having absolutely no biological or other interest. Today several derivatives are deeply examined in medicinal and biological laboratories, and so on, and on.

So, when I asked my long time colleague and friend Vladimir (Volodya) Khripach during the European Chemical Congress in 2012 "Shall we do the Isoprenoids again ?", he responded that he already had calls for it e.g. from Sergazy Adekenov and many others. So we said yes. After this, we wrote to many prominent long period Isoprenoid attendees and got just almost homologous answer: "It was great pity the Isoprenoids were set to sleep, if you have the courage to do it, do the conference again.", and so we did.

As the result you have in front of you the same number of participants as in the final years of the "ancient" Isoprenoids with over 40 colleagues who participated in the Isoprenoids before, with several people who were at all or close to all conferences in this serial of meetings, with the Abstract book printed in the same journal as was the Czech tradition before Białowieża, and moreover, with the special issue of Steroids, devoted to Isoprenoids. Could we wish more?

After almost 50 years after its origin, Isoprenoids are not dead, long live the Isoprenoids.

Pavel Drašar

PLENARY LECTURES

CANNABINOIDS REVISITED: NEW CHEMISTRY, TARGETS AND PLANT SOURCES

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The pharmacological potential of Cannabis (*Cannabis sativa* L.) has long been reductively identified with the one of Δ^9-tetrahydrocannabinol (THC), its psychotropic constituent. While undoubtedly a major player for the medicinal exploitation of Cannabis, THC is not the only constituent of this plant having pharmacological potential, nor cannabinoids are the only class of bioactive compounds present in this plant. These issues are now well established, but less known is the fact that cannabinoids as well as compounds with cannabinoid activity are not unique to Cannabis, but also occur in other plants[1]. Compounds with alleged cannabinoid activity have also been found in food plants and spices.

Cannabinoids are the result of the biogenetic merging of a polyketide-derived alkylresorcinol and an isoprenyl residue[2]. This leitmotiv is not uncommon in natural products, being documented in both lower (liverworts) and higher plants[1]. In hemp cannabinoids, the isoprenyl residue is, with a single exception[3], of the monoterpenyl type, while the resorcinyl alkyl residue has five, or more rarely, a lower number of carbons[2]. However, a large and diverse group of "cannabinoids" also occurs in South-African members of the genus *Helichrysum*. While cannabigerol (CBG) and its acidic precursor (pre-CBG) have both been isolated from *H. umbraculigerum* Less.[4], most *Helichrysum* cannabinoids are derived from the phenethyl version of these compounds, where a phenyl ring replaces the three terminal carbons of the classic pentyl chain of hemp cannabinoids. Compounds of this type have also been isolated from liverworths[1]. Surprisingly, the biological profile of phenethyl cannabinoids is totally unknown, despite their close similarity to hemp cannabinoids and the use of some *Helichrysum* species to induce a trance state not unlike the one associated to the recreational use of hemp. The synthesis and the potential of *Helichrysum* cannabinoids to affect the metabotropic (CBs), ionotropic (TRPs) and trascription factors (PPARs) end-points of hemp cannabinoids will be discussed.

From a pharmacological standpoint, cannbinoids are compounds capable to interact with the metabotropic cannabinoid receptors CB_1 and CB_2. While CB_1 is rather selective in terms of natural products ligands, CB_2 is more promiscuous, and can be modulated by different structural classes of natural products[2]. The most surprisingly ligand of CB_2 is, undoubtedly, the sesquiterpene β-caryophyllene, the only hydrocarbon capable to bind in a specific and potent (nanomolar) way a (non-olfactory) macromolecular biological end-point, and structure-activity relationships for this interaction will be discussed[5], highlighting the potential of the caryophyllane sesquiterpene framework to modify various end-points of endogenous eicosanoids[5]. While β-caryophyllene is a major constituent of the essential oil from hemp, this plant also contains a host of bioactive and unique phenolics (cannflavins, canniprene, cannabispiranone, denbinobin) capable to interact with inflammatory targets and especially the enzymatic formation of eicosanoids (PGs, LTs)[6]. This inhibitory activity on the production of inflammatory eicosanoids complements the mimicry of regulatory eicosanoids (anandamide 2AG) typical of cannabinoids, qualifying Cannabis as a source of compounds capable to selectively modulate endolipid signaling and beneficially affect its pathological imbalance in several pathological settings[7].

REFERENCES

1. Woelkart K., Salo-Ahen O. M., Bauer R.: Curr. Top. Med. Chem. *8*, 173 (2008).
2. Appendino G., Chianese G., Taglialatela-Scafati O.: Curr. Med. Chem. *18*, 1085 (2011).
3. Pollastro F., Taglialatela-Scafati O., Allrà M., Munoz E., Di Marzo V., De Petrocellis L., Appendino G.: J. Nat. Prod. *74*, 2019 (2011).
4. Bohlmann F., Hoffmann, E.: Phytochyemistry *18*, 1371 (1979).
5. Chicca A., Caprioglio D., Minassi A., Petrucci V., Appendino G., Taglialatela-Scafati O., Gertsch J.: ACS Chem. Biol. in print; DOI: 10.1021/cb500177c.
6. Sánchez-Duffhues G., Calzado M. A., de Vinuesa A. G., Caballero F. J., Ech-Chahad, A., Appendino G., Krohn K., Fiebich B. M., Muñoz E.: Biochem. Pharmacol. *76*, 1240 (2008).
7. Werz O., Seegers J., Schaible A. M., Weinigel C., Barz D., Koeberle A., Allegrone G., Pollastro F., Zampieri L., Grassi G., Appendino G.: PharmaNutrition *2*, 53 (2014).

STRIGOLACTONES - A NEW GENERATION OF ISOPRENOID PLANT HORMONES

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The newly identified group of plant hormones strigolactones (SLs) plays an important role in the regulation of shoot branching/tillering and root architecture in plants. In addition to this internal plant signalling function SLs are germination stimulants for root parasitic plant species of the Orobanchaceae, and chemical signals stimulating plant root colonization by symbiotic arbuscular mycorrhizal (AM) fungi. The biosynthetic pathway of SLs has been partially elucidated, using highly branched/tillered mutants of Arabidopsis (max),

rice (dwarf or htd), Petunia (dad) and pea (rms). Using these mutants it was shown that the carotenoid isomerase D27, the carotenoid cleavage dioxygenases CCD7 and CCD8 (in Arabidopsis called MAX3 and MAX4) and a cytochrome P450 (MAX1 in Arabidopsis) are involved in strigolactone biosynthesis. An F-box protein MAX2 and an α/β hydrolase D14 seem to be involved in strigolactone perception/downstream signaling. The role of strigolactones in the regulation of several biological processes, the regulation of their biosynthesis and the current knowledge of their biosynthetic pathway will be discussed.

TARGETING THAPSIGARGIN TOWARDS TUMORS

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The Mediterranean plant *Thapsia garganica* (Apiacae) has for millennia been used in traditional medicine. The skin irritating principle was isolated, named thapsigargin (**I**) and the structure elucidated (Fig. 1)[1]. Thapsigargin is a potent inhibitor of the sarco- or endoplasmatic reticulum Ca^{2+} ATPAase (SERCA). Inhibition of this enzyme induces apoptosis in all kind of cells. This general toxicity of the compound prevents the use of the compound *per se* consequently a produg approach was chosen[2].

The chemistry of **I** was studied in order to be develop methods for selective substitution of the acyl groups and to enable transformations revealing the structure activity relationships. By combing the results from the SAR-studies and the results from a GRID analysis of the binding site as revealed from an X-ray study of the complex of SERCA and **I** led to development of a pharmacophore model. Recent results have revised this model by including the effects of water molecules included in the binding pocket[3]. The pharmacophore model implies that substitution of the butanoyl group with long flexible groups such as 12-aminododecanoyl will inhibit SERCA as efficient as **I**.

Advantage was taken of the recent discovery that the proteolytic enzyme prostate specific membrane antigen (PSMA) is not only found in the prostate but also in neovascular tissue of a number of tumours[4]. The enzyme has very unusual substrate specificity by preferentially cleaving peptides formed by conjugating the γ-carboxylic groups of glutamic acid with the α-amino group of another amino acid (see Fig. 1). However, the presence of the active site in a cave within the enzyme necessitates a linker between the cleavage site and the peptide bond. By substitution of the butanoyl group of **I** with a 12-aminododecaonic acid and conjugating this compound with a peptide analogue **II** was obtained. Incubation of **II** with cancer different malign cell lines revealed that the drug affected only PSMA producing cell lines. A mouse xenograft model showed that growth of LNCaP tumours were significantly inhibited by **II** without any apparent toxic effects.

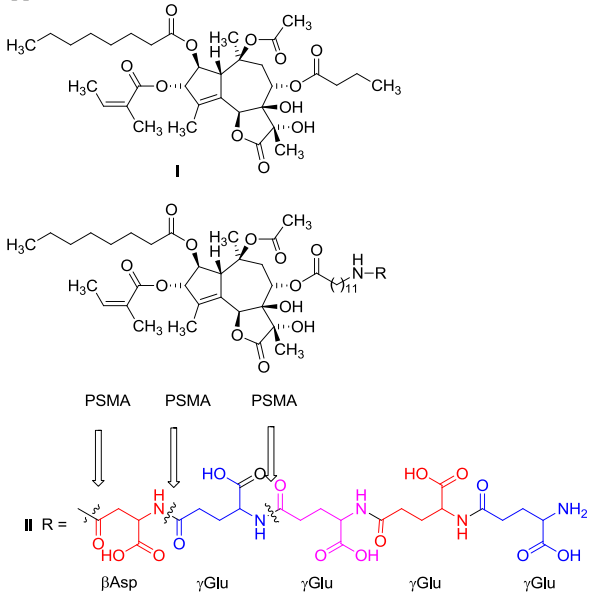

Fig. 1. **Thapsigargin (I) and the prodrug G202 (II). The open arrows indicate the peptide bonds cleaved by PSMA**

A number of toxicity studies in animals were performed and based on a positive outcome of these FDA accepted that clinical trials of **II** were initiated. At the present clinical phase II has been initiated.

Support for the Danish Cancer Society and the Danish Research Council for Strategic Research is acknowledged.

REFERENCES

1. Christensen S. B., Andersen A., Smitt U. W.: Progr. Chem. Natl. Prod. *71*, 131 (1997).
2. Christensen S. B., Skytte D. M., Denmeade S. R., Dionne C., Møller J. V., Nissen P., Isaacs J. T.: Anti-Cancer Agents Med. Chem. *9*, 276 (2009).
3. Paulsen E. S., Villadsen J., Tenori E., Liu H., Bonde D. F., Lie M. A., Bublitz M., Olesen C., Autzen H. E., Dach I., Sehgal P., Nissen P., Møller J. V., Schiøtt B., Christensen S. B.: J. Med. Chem. *56*, 3609 (2013).
4. Denmeade S. R., Mhaka A. M., Rosen D. M., Brennen W. N., Dalrymple S., Dach I., Olesen C., Gurel B., Demarzo A. M., Wilding G., Carducci M. A., Dionne C. A., Møller J. V., Nissen P., Christensen S. B., Isaacs J. T.: Sci. Transl. Med. *4*, 140ra86 (2012).

CHEMICAL BIOLOGY APPLICATIONSFOR CLICK CHEMISTRY IN THE OXYSTEROL AND STEROID FIELDS

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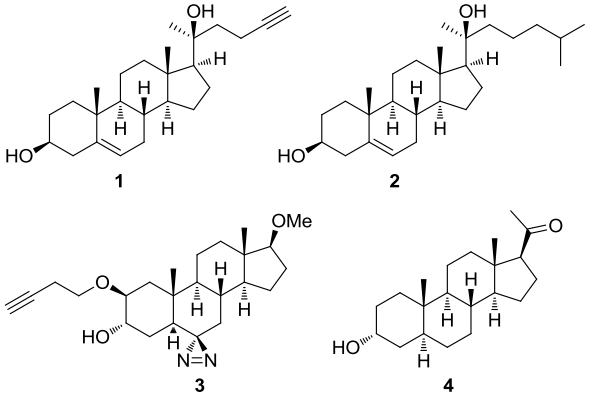
The Huisgen 1,3-dipolar cycloaddition reaction between a terminal alkyne and an azide, commonly referred to as the "click reaction", is now a widely used chemical biology tool. In the sterol/steroid field, it has been investigated for many different uses. In this presentation I will describe the click chemistry we developed for collaborative studies in two different areas of sterol/steroid research.



The first application involves the preparation of an oxysterol alkyne analogue (1) of 20(S)-hydroxcholesterol (2) to investigate the mechanism whereby oxysterols activate the Sonic Hedgehog signaling pathway. This pathway is important during development and also a target for cancer chemotherapy. Secondarily, oxysterol alkyne 1 was also used to gain information about the trafficking of oxysterol 2 in fibroblasts. Little is currently known about how oxysterol movement in cells is regulated. The second area involves the preparation and use of a neurosteroid alkyne analogue (3) of the endogenous neurosteroid allopregnanolone (4) to investigate the distribution of neurosteroid modulators of GABAA receptors in the cellular compartments of neurons, nd to identify the binding sites for neurosteroids on GABAA receptors. Neurosteroid analogues are currently of widespread interest as potential new intravenous general anesthetics and as potential drugs for the treatment of epilepsy, autism and psychiatric diseases.

THE OTHER HEXAGON: CYCLOHEXANE SCAFFOLDS IN SUPRAMOLECULAR CHEMISTRY

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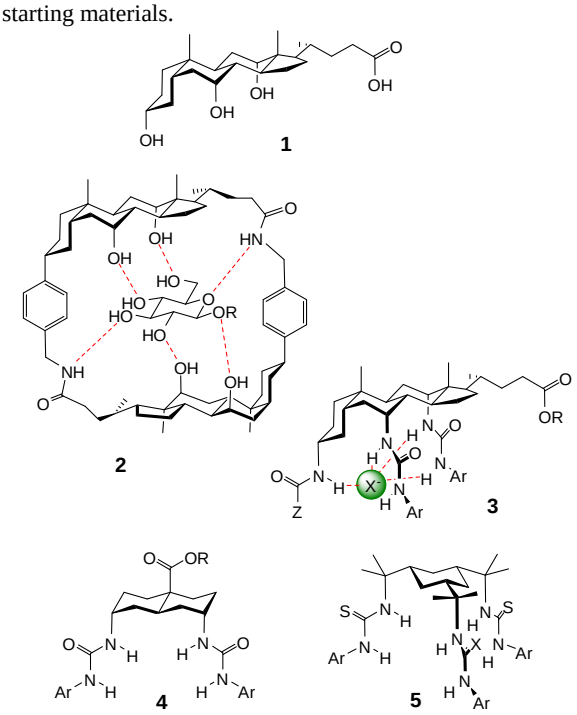
The design of functional molecules requires frameworks with predictable structures, and these are most easily assembled from rigid components. The benzene ring is an obvious and readily available rigid component and, accordingly, this has played a large part in the development of supramolecular chemistry. However, benzene is not the only hexagonal molecule with a well-defined, reliable 3D geometry. Cyclohexanes have a strong preference for chair conformations and, unlike benzene, offer two orientations (axial and equatorial) at each carbon. Moreover the cyclohexane nucleus is common in natural products, especially isoprenoids, and this offers a useful source of starting materials.



In a programme spanning 2-3 decades, our group has developed a range of supramolecular systems which employ cyclohexane-based frameworks. Much of the work has exploited cholic acid **1** as a building block[1]. This inexpensive steroid has been used to create carbohydrate receptors such as **2**[2] and anion receptors such as **3**[3], as well as facial amphiphiles, combinatorial libraries, synthetic enzymes etc. More recently we have realised that synthetic frameworks, inspired by nature but not derived therefrom, can also have advantages. Both decalins **4**[4] and monocycles **5**[5] have performed well as anion binding units.

Perhaps the most important result from this work has been the discovery that anion receptors **3**–**5** are also capable of transmembrane anion transport[6]. Molecules with this function are not readily available from nature, so novel applications may be envisaged. In particular, there is potential for treating conditions resulting from defective anion transport using "channel replacement therapy"[7]. One such condition is cystic fibrosis, a genetic disease which affects many thousands world-wide. The development and optimisation of these

transporters (anionophores) will be a focus of the lecture. A second topic, illustrating the versatility of these scaffolds, will be the exploitation of steroidal frameworks in crystal engineering[8]. A subset of bis-ureiodo cholanoates form nanoporous cystal structures with extraordinary variability. By modifying these structures, and especially by mixing to give organic alloys, it is possible in principle to create materials with a wide range of properties and functions.

REFERENCES

1. Davis A. P.: Chem. Soc. Rev. *22*, 243 (1993) ; Davis A. P.: Molecules *12*, 2106 (2007).
2. Davis A. P., Wareham R. S.: Angew. Chem., Int. Ed. *38*, 2978 (1999).
3. Davis A. P., Joos J.-B.: Coord. Chem. Rev. *240*, 143 (2003); Davis A. P.: Coord. Chem. Rev. *250*, 2939 (2006); Brotherhood P. R., Davis A. P.: Chem. Soc. Rev. *39*, 3633 (2010).
4. Hussain S., Brotherhood P. R., Judd L. W., Davis A. P.: J. Am. Chem. Soc. *133*, 1614 (2011).
5. Cooper J. A., Street S. T. G., Davis A. P.: Angew. Chem., Int. Ed. *53*, 5609 (2014).
6. Koulov A. V., Lambert T. N., Shukla R., Jain M., Boon J. M., Smith B. D., Li H. Y., Sheppard D. N., Joos J. B., Clare J. P., Davis A. P.: Angew. Chem., Int. Ed. *42*, 4931 (2003); McNally B. A., Koulov A. V., Smith B. D., Joos J. B., Davis A. P.: Chem. Commun., 1087-1089 (2005); McNally B. A., Koulov A. V., Lambert T. N., Smith B. D., Joos J. B., Sisson A. L., Clare J. P., Sgarlata V., Judd L. W., Magro G., Davis A. P.: Chem. Eur. J. *14*, 9599 (2008); Valkenier H., Davis A. P.: Acc. Chem. Res. *46*, 2898 (2013).
7. Davis A. P., Sheppard D. N., Smith B. D.: Chem. Soc. Rev. *36*, 348 (2007).
8. Sisson A. L., del Amo Sanchez V., Magro G., Griffin A. M. E., Shah S., Charmant J. P. H., Davis A. P.: Angew. Chem., Int. Ed. *44*, 6878 (2005); Natarajan R., Charmant J. P. H., Orpen A. G., Davis A. P.: Angew. Chem., Int. Ed. *49*, 5125 (2010); Natarajan R., Magro G., Bridgland L. N., Sirikulkajorn A., Narayanan S., Ryan L. E., Haddow M. F., Orpen A. G., Charmant J. P. H., Hudson A. J., Davis A. P.: Angew. Chem., Int. Ed. *50*, 11386 (2011); Natarajan R., Bridgland L., Sirikulkajorn A., Lee J. H., Haddow M. F., Magro G., Ali B., Narayanan S., Strickland P., Charmant J. P. H., Orpen A. G., McKeown N. B., Bezzu C. G., Davis A. P.: J. Am. Chem. Soc. *135*, 16912 (2013).

BRASSINOSTEROID SIGNALLING AT THE PLASMA MEMBRANE: INVOLVEMENT OF ION CHANNELS

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Brassinosteroids form an important class of plant hormones with a multitude of functions. They have been intensively investigated for their biosynthesis, distribution and physiological functions. A number of studies have dealt with identification of brassinosteroid receptors in plant cells. Brassinosteroids have been shown to be perceived at the cell surface by BRI1, leucine-rich repeat receptor-like kinases (LRR RLKs)[1,2]. The cytoplasmic domain of BRI1 probably interacts with calmodulin in a Ca^{2+}-dependent manner, suggesting a cross-talk between brassinosteroid and calcium signalling pathways[3]. Moreover, recent data indicate that brassinosteroids can also be sensed by the plasma membrane system catalysing increase in the cytosolic free Ca^{2+} (in leaves of *Arabidopsis thaliana*)[4]. The aim of this study was to examine possible effects of brassinosteroids on the plasma membrane cation conductances in plant cells.

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), epibrassonolide, homobrassionolide or 24-epicastasterone (10^{-7} – 10^{-4} M) were applied exogenously. Only 24-epicastosterone modified transmembrane cation currents while epibrassonolide and homobrassionolide did not cause any reaction. Addition of 24-epicastosterone at cytosolic side through the patch-clamp pipette led to increase of 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 antagonists of nonselective Ca^{2+}-permeable cation channels. Blockers of K^+ channels did not inhibit this conductance.

The plasma membrane conductance, which was activated by an endogenous or exogenous 24-epicastosterone, showed bell-like shape with maximal activation at depolarisation voltages (bath: 20 mM Ca^{2+}). It was not observed at lower extracellular Ca^{2+}. This demonstrates that the observed conductance was mediated by Ca^{2+} entry through cation channels from extracellular space.

The depolarisation-activated Ca^{2+} channels have rarely been observed in the plasma membrane of higher plants[5-8]. They are usually masked by large outwardly rectified currents or hyperpolarisation activated currents. The genetics of these channels and their regulation remain unclear. It is known that they are stimulated by microtubule-depolymerizing drugs and inhibited by gadolinium ions[5,6]. Zhao *et al*. have recently observed that brassinosteroid-induced elevation of cytosolic free Ca^{2+} was significantly lower in the *bri1-5* plants having reduced sensitivity to brassionosteroids[9]. Thus, the brassinosteroid-induced Ca^{2+} signal is probably located downstream of brassinosteroid binding to BRI1 receptor.

Surprisingly, the activation of Ca^{2+}-permeable channels was not observed after application of epibrassonolide and homobrassionolide to wheat root protoplasts. Hypothetically, this can be related to the absence of the biosynthetic path

from 24-epicastosterone to epibrassinolide in gramenaceous species[10].

Here we also tested the brassinosteroid effect on cytosolic free Ca^{2+}, using *Arabidopsis thaliana* plants constitutively expressing aequorin. All three brassionosteroids induced elevation of the cytosolic free Ca^{2+} in arabidopsis root cells with 24-epicastosterone being more potent than epibrassonolide and homobrassionolide. Minimal concentration of 24-epicastosterone, which induced statistically significant changes of cytosolic free Ca^{2+}, was $3*10^{-6}$ M.

In conclusion, the obtained results indicate that the plasma membrane of root cells contains brassinosteroid-activated Ca^{2+}-permeable cation channels, which are involved in the generation of cytosolic Ca^{2+} signal.

REFERENCES
1. Shiu S.H., Karlowski W.M., Pan R., Tzeng Y.H., Mayer K.F., Li W.H.: Plant Cell *16*, 1220 (2004).
2. Belkhadir Y., Chory J.: Science *314*, 1410 (2006).
3. Oh M.H., Kim H.S., Wu X., Clouse S.D., Zielinski R.E., Huber S.C.: Biochem J. *443*, 515 (2012).
4. Zhao Y., Qi Z., Berkowitz G.A.: Plant Physiol. *163*, 555 (2013).
5. Miedema H., Demidchik V., Véry A.A., Bothwell J.H.F., Brownlee C., Davies J.M.: New Phytol. *179*, 378 (2008).
6. Thion L., Mazars C., Nacry P., Bouchez D., Moreau M., Ranjeva R., Thuleau P.: Plant J. *13*, 603 (1998)
7. Thuleau P., Ward J.M., Ranjeva R., Schroeder J.I.: EMBO J. *13*, 2970 (1994).
8. Demidchik V., Davenport R.J., Tester M.A.: Ann. Rev. Plant Biol. *53*, 67 (2002).
9. Wang X., Goshe M.B., Soderblom E.J., Phinney B.S., Kuchar J.A., Li J., Asami T., Yoshida S., Huber S.C., Clouse S.D.: Plant Cell *17*, 1685 (2005).
10. Kim B.K., Fujioka S., Takatsuto S., Tsujimoto M., Choe S.: Biochem. Biophys. Res. Commun. *374*, 614 (2008).

STEREOCHEMICAL CONTROL IN THE NATURAL PRODUCTS SYNTHESIS: SOLANOECLEPIN A AND WITTIG REARRANGEMENTS

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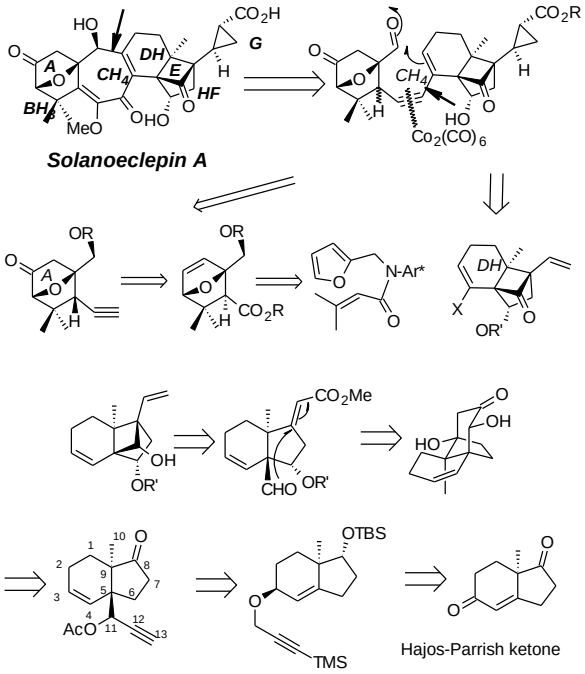
We have been studying stereochemical control for natural product synthesis such as solanoeclepin A (1), for which we have recently achieved the partial syntheses aiming at the cyclobutane-ring formation via the following retrosynthetic route from *ent*-Hajos-Parrish ketone via [2,3]-Wittig rearrangement, and radical cyclization through aldehyde-enone. The Left Segment was recently synthesized via intramolecular Diels-Alder cycloaddition. After coupling with a C-ring model, the Prins-carbonyl-ene cyclization with the acetylene dicobalt complex gave the seven membered product using a Lewis acid catalyst. The resulting stereochemistry of the sec-hydroxyl group can be beta-selective but the double bond control has to be waited for improvement. During the synthesis, we have explored a synthesis of *trans*-C/D ring system, which was generally very difficult to obtain. The key reaction is [2,3]-Wittig rearrangement, which has been known since long time ago. Recently the rearrangement on allyl-propargyl ether on dihydropyrane ring was studied to find unique mechanism, which will be also discussed as the general methodology for the synthesis of other natural products.



REFERENCES
1. Tsao K.-W., Isobe M.: Org. Lett. *12*, 5338 (2010).
2. Isobe M., Niyomchon,, S., Cheng C-Y., Hasakunpaisarn A.: Tetrahedron Lett. 52, 1847 (2011).
3. Tsao K.-W., Cheng C.-Y., Isobe M.: Org. Lett. *14*, 5274 (2012).
4. Isobe M., Kitamura M., Goto T.: Tetrahedron Lett. *21*, 4727 (1980).
5. Isobe M., Huang K.-H.: Manuscript in preparation.
6. Isobe M.: Pure & Appl. Chem. *85*, 1149 (2013).

CATALYTIC ASYMMETRIC SYNTHESIS OF ISOPRENOIDS WITH QUATERNARY STEREOCENTERS

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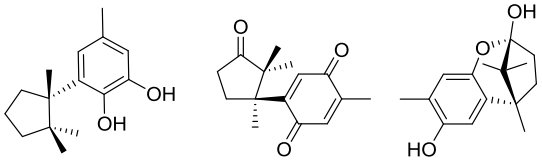
The catalytic asymmetric construction of quaternary stereocenters, tertiary alcohols, and ethers is widely regarded

as one of the major challenges in synthetic organic chemistry[1]. For quaternary stereocenters, one efficient strategy to achieve this goal is the conjugate addition of carbon nucleophiles to β,β-disubstituted unsaturated carbonyl compounds[2]. Employing asymmetric copper catalysis, the groups of Alexakis, Fillion and Hoveyda successfully added trialkylaluminum, dialkylzinc and alkyl Grignard reagents to a variety of β,β-disubstituted unsaturated electrophiles[3]. Though addition of the corresponding arylaluminum and arylzinc reagents was feasible, the general use of aryl Grignard reagents is still problematic due to their high reactivity. Alternatively, rhodium-catalyzed conjugate addition of organoboron reagents, developed by Hayashi and co-workers, has been frequently employed[4,5].

In 2011, the Stoltz laboratory reported the first asymmetric palladium-catalyzed conjugate addition of arylboronic acids to cyclic β-disubstituted enones using *t*BuPyOx as the ligand[6]. Shortly thereafter we reported the asymmetric Michael addition of arylboronic acids to cyclic β-disubstituted enones and lactones, catalyzed by $PdCl_2$-(*R,R*-PhBOX)[7]. Very recently Sigman and co-workers reported the asymmetric Pd-catalyzed remote benzylic quaternary stereocenter formation using arylboronic acids[8]. A broad substrate scope, consisting exclusively of linear substrates, was reported with very good to excellent enantioselectivities and yields.

In the lecture, I will discuss our contribution to the development of the asymmetric palladium-catalyzed conjugate addition of arylboronic acids to cyclic β-disubstituted enones, with an emphasis on its application in the synthesis of α-cuperenone, herbertenediol, enokipodin A and enokipodin B[9].

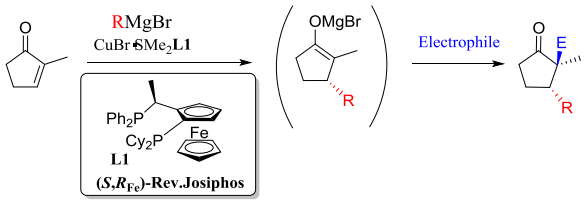


Herbertenediol **Enokipodin B** **Enokipodin A**

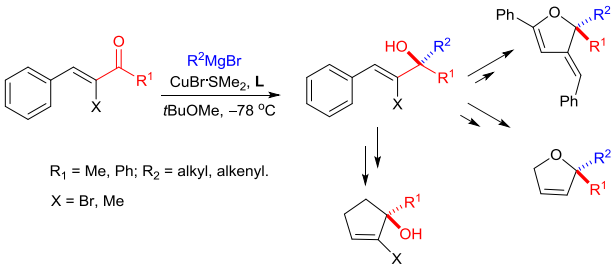
An alternative approach is the asymmetric conjugate addition to Michael acceptors possessing α-substitution, followed by alkylation of the (regioselectively) formed enolate.

Until very recently, the sole report in this field came from Vuagnoux-d'Augustin and Alexakis, and comprized the enantioselective addition of Me_3Al and Et_3Al to 2-methyl cyclohexenone[10]. This knowledge was subsequently used by Helmchen *et al.* in a synthesis of pumiliotoxin C[11]. This was followed by Mauduit, Alexakis *et al.* who reported the successful application of Cu(I)-*N*-heterocyclic carbene complexes in the assymetric addition of Grignard reagents to 2-methyl cyclopentenone and –hexenone[12]. The resulting magnesium enolates were subsequently alkylated to provide a quaternary stereocenter vicinal to the initially formed tertiairy stereocenter.

In the lecture, I will discuss our contribution to the asymmetric copper-catalyzed conjugate addition of Grignard reagents to 2-methyl cyclopentenone and the subsequent enolate alkylation with a variety of electrophiles[13].



The catalysed addition of organometallic reagents to ketones is in principle one of the most straightforward methods for the synthesis of chiral enantiopure tertiary alcohols. Recently, we reported on the use of a copper/Josiphos-type catalyst system to accomplish the enantioselective 1,2-addition of Grignard reagents to α,β-unsaturated ketones[14-16]. This leads to chiral enantioenriched tertiary allylic alcohols, although currently β-branched Grignard reagents are required for high enantioselectivity. We showed that this approach can be used for the synthesis of five-membered oxygen heterocycles that function as building blocks for natural product synthesis[17]. Currently we are studying the application of this methodology in natural product synthesis



REFERENCES

1. Christoffers J., Baro A. (eds.): *Quaternary Stereocenters: Challenges and Solutions for Organic Synthesis*, Wiley-VCH, Weinheim, 2005.
2. Alexakis A., Krausse N., Woodward S. in: *Copper-catalyzed Asymmetric Synthesis*, (Alexakis A., Krausse N., Woodward S., ed.) Wiley-VCH, Weinheim, 2014; chapter 2.
3. McGrath K.P., Hoveyda A. H.: Angew. Chem. Int. Ed., *53*, 1910 (2014), and references cited therein.
4. Mauleón P., Carretero J. C.: Chem. Commun. *2005*, 4961.
5. Hahn B. T., Tewes F., Fröhlich R., Glorius F.: Angew Chem., Int. Ed., *49*, 1143 (2010).
6. Kikushima K., Holder J. C., Gatti M., Stoltz B. M.: J. Am. Chem. Soc. *133*, 6902 (2011).
7. Gottumukkala A. L., Matcha K., Lutz M., de Vries J. G., Minnaard A.: J. Chem. Eur. J. *18*, 6907 (2012).
8. Mei T.-S., Pater H. H., Sigman M. S.: M. S. Nature 2014, DOI: 10.1038/nature13231.
9. Buter J., Moezelaar R., Minnaard A.: J. Org. Biomol. Chem. (2014), DOI: 10.1039/c4ob01085j.
10. Vuagnoux-d'Augustin M., Alexakis A.: Chem. Eur. J. *13*, 9647 (2007).
11. Gärtner M., Qu J., Helmchen G.: J. Org. Chem. *77*, 1186 (2012).
12. Germain N., Guénée L., Mauduit M., Alexakis A.: Org. Lett. *16*, 118 (2014).

13. Calvo B. C., Madduri A. V. R., Harutyunyan S. R., Minnaard A. J.: Adv. Synth. Catal. *356*, 2061 (2014).
14. Madduri A. V. R., Harutyunyan S. R., Minnaard A. J.: Angew. Chem. Int. Ed. *51*, 3164 (2012).
15. Madduri A. V. R., Minnaard A. J., Harutyunyan S. R.: Chem. Commun. *48*, 1478 (2012).
16. Madduri A. V. R., Minnaard A. J., Harutyunyan S. R.: Org. Biomol. Chem. *10*, 2878 (2012).
17. Wu Z., Madduri A.V.R., Harutyunyan S. R., Minnaard A. J.: Eur. J. Org. Chem. *2014*, 575.

TOTAL SYNTHESIS OF 1α,25-DIHYDROXYVITAMIN D_3 (CALCITRIOL) AND ANALOGUES

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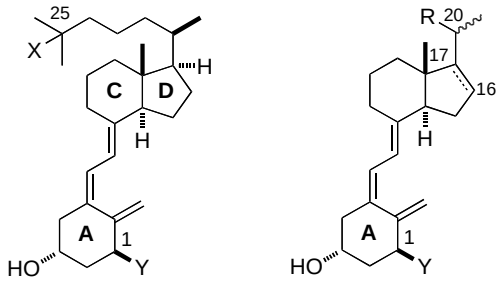
1α,25-Dihydroxyvitamin D_3 [***Ia***, 1α,25-$(OH)_2$-D_3, 1,25D, calcitriol, Figure 1], the active form of the secosteroid vitamin D_3 (***Ib***), interacts with the vitamin D nuclear receptor (VDR) to control mineral homeostasis and multiple cellular processes including differentiation, anti-proliferation, growth, apoptosis, angiogenesis and inmunomodulation[1]. The hormone 1α,25-$(OH)_2$-D_3 is therapeutically used to treat renal failure, risckets, and hyperparathyroidism. Unfortunately, the therapeutic application of 1α,25-$(OH)_2$-D_3 as an antitumor agent has been severely limited by its hypercalcemic effects. Efforts to develop analogues with selective properties, in particular with low or negligible calcemic effects, for treatment of cancer and skin diseases have led to more than 3000 analogues, although only a few have reached the pharmaceutical market or advanced clinical trials[2,3].

Most of the synthesized analogues of 1α,25-$(OH)_2$-D_3 are functionalized at the side chain or **A**-ring. The most common synthetic approaches employed start from vitamin D_3 or vitamin D_2 degradation intermediates containing **CD**-side chain fragments. Because of synthetic difficulties, only a few analogues modified at the **CD**-rings have been developed[4-6].



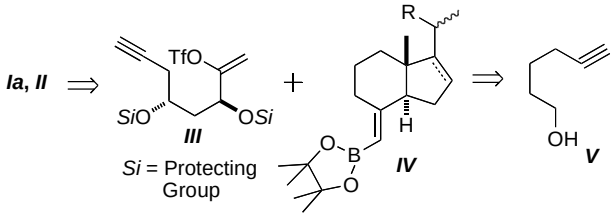
Ia, X=Y=OH
Ib, X=Y=H

II

Fig. 1. **Structures of 1α,25-$(OH)_2$-D_3 (*Ia*) vitamin D_3 (*Ib*) and target analogues II.**

We describe here a versatile total synthesis of 1α,25-$(OH)_2$-D_3 (***Ia***) and their analogues ***II***, which contain either a C16-C17-double bond at the **D**-ring or an epi-configuration at C20. These two types of analogues have proven to induce high cell differentiation and are therefore of potential interest as antitumor agents.

Our plan for the total synthesis of 1α,25-$(OH)_2$-D_3 and target analogues ***II*** is shown in Scheme 1. The construction of the vitamin D triene system is based on a Pd(0)-catalyzed intramolecular cyclization-Suzuki-Miyaura coupling cascade recently developed in our laboratories.[5] The required boronate ***IV*** is prepared from commercial 5-hexyn-1-ol (***V***) utilizing a new method for the installation of angular all carbon quaternary stereocenters that will be described in detail.



Scheme 1. **Retrosynthesis of 1α,25-$(OH)_2$-D_3 (*Ia*) and analogues *II*.**

We thank the Spanish Ministry of Economy and Innovation (MEI, SAF2010-15291), Xunta de Galicia (projects CN2012/074 and INCITE08PXIB209130PR) for financial support. BL thanks the Spanish MEI for an FPI fellowship.

REFERENCES

1. Feldman D., Pike J. W., Adams J. S. (Eds.) *Vitamin D*, Third ed., Elsevier Academic Press, San Diego, CA, 2011.
2. Plum L. A, DeLuca H. F.: Nat. Rev. Drug. Discov. *9*, 941 (2010).
3. Bouillon R., Okamura W. H, Norman W. H.: Endocr. Rev. *16*, 200 (1995).
4. Chapelon A. S., Moraléda D., Rodríguez R., Ollivier C., Santelli M.: Tetrahedron *63*, 11511 (2007).
5. Kabat M. M., Radinov R.: Curr. Opin. Drug Discov. Dev. *4*, 808 (2001); Krause S., Schmalz H.-G. in: *Organic Synthesis Highlights IV*, Ed. Schmalz H.-G., Wiley-VCH, Weinheim, 2000.
6. Zhu G. D., Okamura W. H.: Chem. Rev. *95*, 1877 (1995).
7. Pranjal G., Sigüeiro R., Eduardo S., Mouriño A.: Chem. Eur. J. *16*, 1432 (2010).

STRUCTURE AND FUNCTION OF ENZYMES RESPONSIBLE FOR BIOSYNTHESIS AND METABOLISM OF CHOLESTEROL DERIVATIVES

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Cytochromes P450 (CYP) are widely distributed in the nature enzymes being found across all biological kingdoms including prokaryotes and eukaryotes. They play an essential role in endogenous biosynthesis of steroids, metabolism of fatty acids and vitamins, eicosanoids, biosynthesis of biologically active compounds in plants and oxidation of xenobiotics in animal species. Cytochrome P450 proteins utilize heme cofactor to perform molecular oxygen activation with subsequent oxidation chemistry of wide diversity of drugs and endogenous molecules. Membrane-bound CYPs localized either in the endoplasmic reticulum or mitochondria, where they use different redox partners to shuttle electrons from NADPH to molecular oxygen, finally resulting in the insertion of one oxygen atom into substrate while reducing the other one to water. The active site of cytochrome P450 is buried in the core of the protein and represents a preformed cavity above the heme connected with the surface through a channel.

There are 57 CYP enzymes expressed in human genome with a wide varieties of functions, classified like all CYPs according to the sequence comparisons where families share similarity more than 40% and subfamilies > 55%. The clinical relevance of cytochrome P450s is in its central role in drug metabolism in human organs and tissues as well as outstanding role that hemeproteins play in biosynthesis of low molecular weight bioregulators such as steroid hormones, bile acids and vitamins. Any abnormalities in functioning of cytochrome P450 enzymes result in severe diseases or uncontrolled function of organs and tissues. Some CYPs exhibit both endogenous and exogenous functionality including steroid biosynthesis and metabolism (CYPs 2,7,11,17,19, 21,51). Despite a unique, but rather conserved P450 fold, the molecular mechanisms of CYP substrate specificity and selectivity remain elusive.

To determine the structural determinants that govern the stereo-specific hydroxylation of cholesterol and its derivatives, we determined the crystal structure of human recombinant CYPs 2R1 (vitamin D3 25-hydroxylase), 7A1 (cholesterol 7α-hydroxylase), 7B1 (oxysterol 7α-hydroxylase), 11A1 (cholesterol side chain cleavage), 11B2 (aldosterone synthase) and 51A1 (lanosterol 14α –demethylase) involved mostly in cholesterol metabolism in ligand-free and ligand-bound forms. The data obtained shed light on structural organization of the hemeprotein active site and allow explain the intrinsic mechanism of cholesterol interaction with the amino acid residues of the active site of cholesterol metabolizing cytochrome P450's and determine the molecular mechanism of stereo-specificity of steroid hydroxylation.

TERPENOIDS 2.0 - NEW WAYS IN THE DISCOVERY AND SYNTHESIS OF BIOACTIVE ISOPRENOIDS

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The organic synthesis of terpenoids and prenylated compounds is principally unlimited in structural design but requires multistep reactions, which are step, cost and time intensive. Hence, the enzymatic synthesis of terpenoids and related compounds is increasingly used as an effective alternative. However, the in vitro use of terpene synthases is not economic because the starting prenyl diphosphates are expensive, and some enzymes also are not well behaved ex vivo. In vivo production is cheaper if enough prenyldiphosphate is produced by the host cells, but is structurally limited to very few selected examples of natural terpenoids, although with the advent of synthetic biology some more terpenoids become accessible.

Can we go beyond the limitations of both, the first generation of (petroleum chemistry based) chemical syntheses of terpenoids; and the first generation of biocatalytic or in vivo fermentative production of complex chiral terpenes from very simple linear achiral precursors? Can we combine the best of both worlds, the one step conversion of enzymes and the unlimited structural variation of chemistry? If it where possible to have a systematic and efficient approach to (almost) any kind of prenyldiphosphate precurser in combination with promiscuous terpene synthases as ideal catalysts, old and new terpenoids can be generated quickly and efficiently. This chemoenzymatic combination approach will give "naturally produced non-natural" terpenoids impossible or very difficult to access via either chemical synthesis or biotechnology alone.

Based on these considerations we studied both, efficient access and biotransformation of artificial prenyl diphosphates. Aromatic prenyltransferases, oligoprenyl, mono and sesquiterpene synthases can show both a high promiscuity (i.e. inselective in substrate permission) and a high liberality (i.e. inselective in products formation from a single substrate). Used with unusual, heterosubstituted substrates this leads to a variety of new terpenoids. Some converted the artificial substrates along the same route as their natural one, leading to similar skeletons, sometimes selectively to one product, sometimes liberally to a family of related products. Interestingly, almost always an artificial substrate could be found which shows better conversion rates than the natural one. In other cases, however, with the artificial substrates totally different cyclization modes are followed, leading to unexpected and even (for the moment) unpredictable new terpenoid structures. Independent of predicted or unpredicted behavior, the combination of unnatural substrate and natural catalyst produces structurally new series of terpenoid structures.

Upscaling of the enzyme assays enabled the structure elucidation of these new terpenoids by GC/MS and NMR spectroscopy.

REFERENCES

1. Wessjohann L. A., Keim J., Weigel B., Dippe M.: Curr. Op. Chem. Biol. *17*, 229 (2013).
2. Wessjohann L. A., Zakharova S., Schulze D., Kufka J., Weber R., Bräuer L., Brandt W.: CHIMIA International J. Chem. *63*, 340 (2009).
3. Brandt W., Bräuer L., Günnewich N., Kufka J., Rausch F., Schulze D., Schulze E., Weber R., Zakharova S., Wessjohann L.: Phytochemistry *70*, 1758 (2009).

KEYNOTE LECTURES

CIRCULAR DICHROISM AS A POWERFUL TOOL IN STEREOCHEMICAL STUDIES OF ENONES

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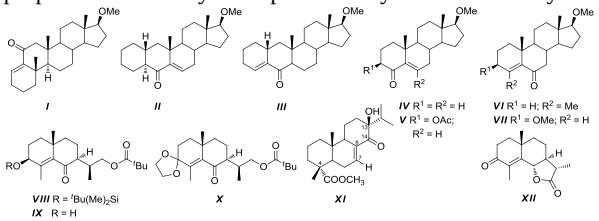
It is well known that, similarly to other groups of biologically active compounds, a wide variety of the medicinal potential of *cis*-enones is strictly related to their absolute configuration. Thus, the relationship between absolute structure and chiroptical properties requires a deeper understanding.

The main objective of this work was to find a correlation between the sign of the nπ* Cotton effect (CE) and the stereochemistry of enone chromophore or its nearest surroundings. To achieve this goal a number of suitable model compounds presented in Scheme 1 were synthesized, and their geometries, structural parameters and chiroptical properties were analysed experimentally and theoretically.



Scheme 1. **Investigated enones I-XII**

To assist in achieving the objectives of the planned study and to obtain the most accurate results, a single crystal X-ray structure determination was applied, where possible. Such an approach enables to specify configurational and conformational chirality by comparing the ECD spectra in the solid phase and solution. A detailed conformational analysis led to finding the predominant conformers. Next, a comparison of the experimental ECD spectra with the spectra simulated by TDDFT calculations provided a reasonable interpretation of the collected data. Ultimately, our study enabled a close examination of the influence of substituents in the vicinity of the chromophore on the conformation and thus the chiroptical properties of the models studied.

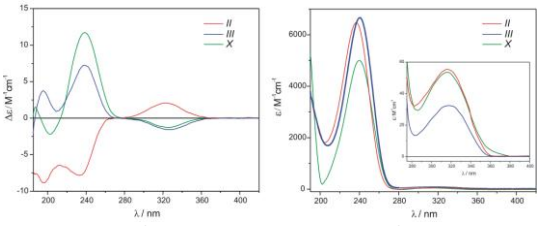


Fig. 1. **ECD (left) and UV (right) spectra of enones II, III, and X recorded in acetonitrile in the 400-190 nm range**

This work was supported by the NCN (Poland), grant UMO-2011/01/B/ST5/06413.

EXPLORING THE REACTIVITY OF STEROID BEARING SPIROKETAL SIDE CHAINS

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Steroid sapogenins ***(I-IV)*** have focused intensive interest for nearly a century due to both, their intrinsic biological activity and usefulness as synthetic starting materials. The exploration of the reactivity of the spiroketal side chain of steroid sapogenins has provided a wide number of transformations that paved the way to the synthesis of a wide variety of bioactive compounds that includes sexual and adrenocortical hormones, plant grow promoting substances and more recently cephalostatins and ritterazines amongst many others.

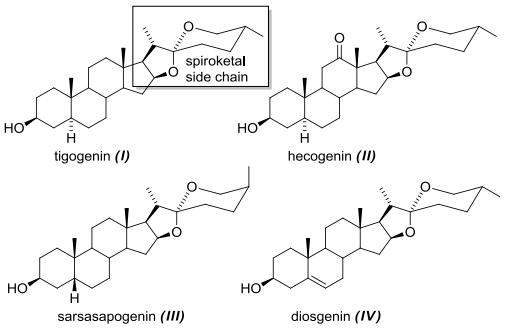
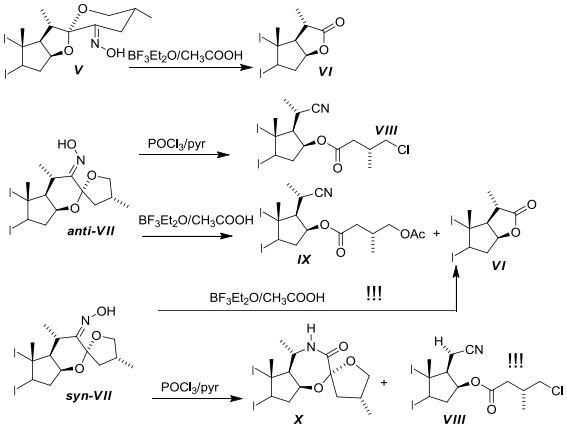


Fig. 1. **Some steroid sapogenins**

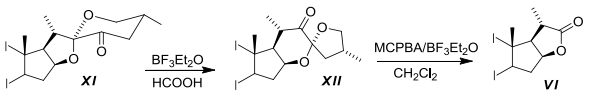
This communication describes some of the recent finding sof our research team on the transformation of steroids bearing different spiroketal moieties in the side chain.



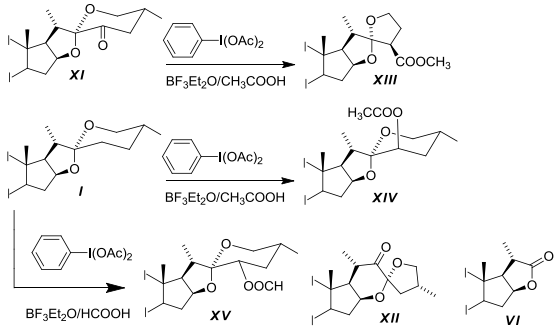
Scheme 1. **Beckmann reactions of 22- and 23-hydroxy-iminospirostanes**

These reactions, which include rearrangements[1-5] (Scheme 1-2) as well as hypervalent iodine-induced

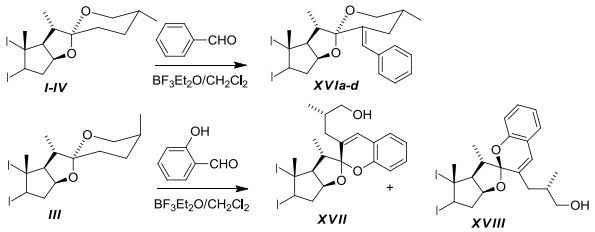
functionalizations (Scheme 3)[6-8], and aldolic condensations (Scheme 4),[9,10] allow the compilation of more information on the particular reactivity of this fragment, and have given rise to compounds of both biological and synthetic interest.



Scheme 2. **Acid-catalyzed reactions of 22 and 23-oxo-sapogenins**



Scheme 3. **Hypervalent iodine-induced trasformations of the spiroketal side chain**



Scheme 4. **Aldol condensations in the spiroketal side chain**

The author acknowledges the financial support provided by "Dirección General de Asuntos del Personal Académico" (Project DGAPA-IN211714) and the Faculty of Chemistry (PAPIIT-5000-9063).

REFERENCES

1. Iglesias-Arteaga M. A.:, Alvarado-Nuño A. A.: Tetrahedron Lett. *47*, 5351 (2006).
2. Macias-Alonso M., Flores-Alamo M., Iglesias-Arteaga M. A.: Steroids *74*, 112 (2009).
3. Macias-Alonso M., Morzycki JW., Iglesias-Arteaga M. A.: Steroids *76*, 317 (2011).
4. Macias-Alonso M., Flores-Alamo M., Iglesias-Arteaga M.A.: Steroids *76*, 1021 (2011).
5. Macias-Alonso M., Flores-Alamo M., Iglesias-Arteaga M. A.: Steroids *78*, 787 (2013).
6. Iglesias-Arteaga M. A., Velazquez-Huerta G. A.: Tetrahedron Lett. *46*, 6897 (2005).
7. Iglesias-Arteaga M. A., Arcos-Ramos R.O.: Tetrahedron Lett. *47*, 8029 (2007).
8. Iglesias-Arteaga M. A., Arcos-Ramos R. O.: Tetrahedron Lett. *48*, 7485 (2007).
9. Ruíz-Pérez K. M., Romero-Ávila M., Tinajero-Delgado V., Flores-Álamo M., Iglesias-Arteaga M. A.: Steroids *77*, 819 (2012).
10. Ramos-Enríquez M. A., Flores-Álamo M., Iglesias-Arteaga, M. A.: Tetrahedron Lett. *54*, 4401 (2013).

CLICK CHEMISTRY IN STEROID AND TERPENE CHEMICAL BIOLOGY TOOLS

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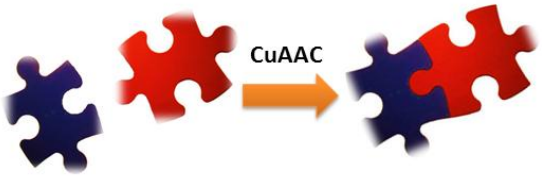
Huisgen copper(I) catalyzed 1,3-dipolar cycloaddition reaction[1,2], taking place between terminal alkynes and azides ("click chemistry", CuAAC), is very often applied to chemical modifications tailored to synthesize substances quickly and reliably by joining small units together[3]. This reaction has been employed in many research areas such as development of new drugs, chemistry of polymers and materials, biology and bioconjugation[4,5]. In our group, we use CuAAC approach to generate derivatives of selected steroids and terpenoids tailored for biochemical and medicinal studies. This contribution summarizes our current results in chemical labeling by fluorescent and/or biotin tag, synthesis of steroid-terpene conjugates and the click-dimerization of steroids.



This work was supported by Czech Science Foundation (GAČR) 303/14/ 04329-S, VG20112015045, and MSMT No 20/2014.

REFERENCES

1. Huisgen R.: Angew. Chem. Int. Ed. *75*, 604 (1963).
2. Huisgen R.: Angew. Chem. Int. Ed. *2*, 633 (1963).
3. Moses J. E., Moorhouse A. D.: Chem. Soc. Rev. *36*, 1249 (2007).
4. Kolb H. C., Sharpless K. B.: Drug. Discov. Today. *8*, 1128 (2003).
5. Kolb H. C., Finn M. G., Sharpless K. B.: Angew. Chem. Int. Ed. *40*, 2004 (2001).

TOTAL SYNTHESIS OF *ENT*-PROGESTERONE

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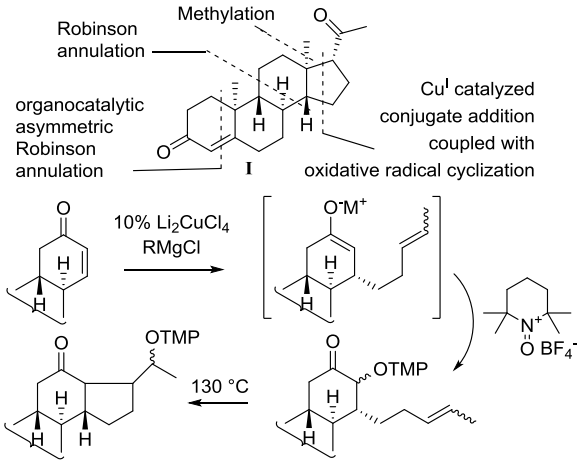
Steroids occur in Nature exclusively in one enantiomeric form, due to conservative biosynthetic pathways. Clearly defined binding pockets in nuclear receptors distinguish between natural (*nat*-) and enantiomeric (*ent*-) steroids. However, some membrane steroid receptors (e.g. $GABA_A$) are affected by *ent*-steroids, probably through membrane perturbation[1]. We will discuss here a total synthesis of *ent*-progesterone **I** (Fig. 1) to probe the mechanism of action of steroids at the NMDA receptor. The retrosynthesis was planned in respect to explore the minimum binding requirement of these substrates at NMDA receptors. Physiological activities of some neurosteroid congeners will be discussed.



Scheme 1. ***ent*-Progesterone retrosynthesis and the key step**

The synthesis starts by construction of the AB decalin ring system by organocatalytic asymmetric Robinson annulation. Substrate-controlled Robinson annulation gives rise to ring C, followed by reduction-oxidation to install the correct enone isomer for the following key step. The precursor of ring D and side chain is attached by a novel reaction sequence consisting of Cu-catalyzed conjugate addition and oxygenation. A thermal radical cyclization employing the persistent radical effect[2] leads to annulation of ring D. Unprecedented stereoselectivity was observed during the cyclization step. Thermodynamically controlled methylation completes the construction of the steroid skeleton.

REFERENCE
1. Covey D. F.: Steroids *74*, 577 (2009).
2. Studer A.: Chem.-Eur. J. *7*, 1159 (2001).

METABOLIC FATE OF 20-HYDROXYECDYSONE AND POSTSTERONE IN RODENTS AND HUMANS

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Dedicated to Karel Slama for his 80th birthday.

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Phytoecdysteroids (plant analogues of insect moulting hormones) display a wide set of pharmacological effects on mammals, but their molecular targets have not yet been identified. Injected or ingested phytoecdysteroids are converted into numerous metabolites[1], only some of which have been identified, and we do not know whether these are active or not.

In mice, the digestive tract always contains most of the ingested 20E. Within 30 minutes after ingestion, 20E reaches the large intestine where microorganisms efficiently remove the 14-hydroxyl group and more slowly cleave the side-chain. Between 1 and 2 hours post-ingestion, a very complex set of metabolites appear, which correspond to poststerone derivatives formed in the liver. 20E and its metabolites undergo an entero-hepatic cycle, involving glucuronide conjugation in the liver and subsequent deconjugation in the (large) intestine. Similar 20E metabolites were observed in mice, rats and humans, among which 20,26-dihydroxy-ecdysone, 6αOH-20E, 14-deoxy-20E, 6αOH-14-deoxy-20E, poststerone and 14-deoxy-poststerone. Poststerone has greater bioavailability than 20E and it undergoes efficient epimerization at C-3 and hydroxylation(s) in the liver.

Ingested 20E undergoes a very complex metabolism in rodents and Humans. These data, together with parallel *in vitro* experiments provide a basis for the identification of 20E metabolite(s) possibly involved in its *in vivo* physiological effects.

The work presented here was co-funded by the BPI; FUI (French State Fund), Ile de France Region and Seine Saint Denis General Councils.

REFERENCE
1. Kumpun S., Girault J.-P., Dinan L., Blais C., Maria A., Dauphin-Villemant C., Yingyongnarongkul B., Suksamrarn A., Lafont R.: J. Steroid Biochem. Mol. Biol. *126*, 1 (2011).

ELECTROSTATICALLY GUIDED DYNAMICS – THE ROOT OF FIDELITY IN PROMISCUOUS TERPENE SYNTHASES?

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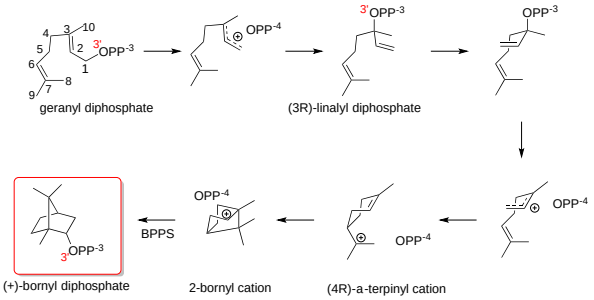
Terpene cyclases are responsible for the initial cyclization cascade in the multistep synthesis of more than 60,000 known natural products. This abundance of compounds is generated using a very limited pool of substrates based on linear isoprenoids. The astounding chemodiversity obtained by terpene cyclases suggests a tremendous catalytic challenge to these often promiscuous enzymes. In the current study we present a detailed mechanistic view of the biosynthesis of the monoterpene bornyl diphosphate (BPP) from geranyl diphosphate by BPP synthase (BPPS) (Scheme 1). We employ molecular dynamics and multidimensional free energy simulations on an accurate hybrid quantum mechanics-molecular mechanics potential energy surface to study the enzymatic pathways. We identify the bornyl cation as a key mechanistic branching point that can form the product BPP as well as the side product camphene. Initial heterolytic C-O bond cleavage in (3R)-linalyl diphosphate yields a linalyl cation, followed by formation of terpinyl and pinyl cations. Subsequently, the bornyl cation is formed via a low, enzyme-induced barrier separating the bornyl cation from the pinyl cation. Importantly, the bornyl cation is not a stable species but serves as a TS between BPP and the camphyl cation. The bornyl cation may be viewed as an enzyme induced bifurcation point on the potential of mean force multidimensional surface. Chemical dynamics simulations suggest that a key factor in assuring BPP formation is electrostatic steering by the diphosphate moiety, which directs reaction trajectories towards product formation (Fig. 1). Nonetheless, chemical dynamics is not precise enough for exclusive product formation, providing a long-sought after rationale for fidelity in this promiscuous terpene cyclase. The current results suggest that terpene cyclases such as BPPS may have evolved to direct product distribution via dynamical effects even though this is a possible reason for their promiscuity[1,2].



Scheme 1. **Reaction mechanism in BPPS**

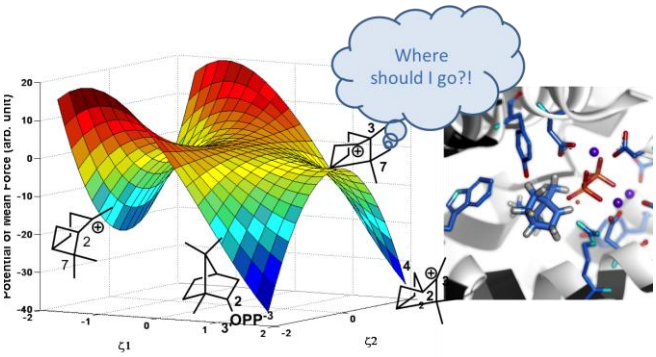


Fig. 1. **Schematic depiction of the bifurcating potential of mean force surface in BPPS**

REFERENCES

1. Weitman M., Major D. T.: J. Am. Chem. Soc. *132*, 6349 (2010).
2. Major D. T., Weitman M.: J. Am. Chem. Soc. *314*, 19454 (2012).

CHEMISTRY AND BIOACTIVITY OF POLYGONUM SESQUITERPENOIDS

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The genus *Polygonum* L. (Polygonaceae), which is well known for its use in the oriental traditional medicine systems for the treatment of various ailments including cancers, fever, inflammation, infections, pain and tumours, comprises ca. 130 species of perennial herbs and shrubs, commonly known as knotweed, knotgrass, bistort, tear-thumb or mile-a-minute. As part of our phytochemical and bioactivity studies on *Polygonum* species, especially from the Bangladeshi flora, several bioactive sesquiterpenes have been isolated and identified. This talk will present an overview on the chemistry and bioactivity of sesquiterpenes found in the genus *Polygonum*, especially highlighting the findings from our own studies.

A NEW LOOK AT THE NATURE OF INSECT JUVENILE HORMONE: 50 YEARS OF EXPERIENCE

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During more than 50-year history of insect juvenile hormone (JH) research, two alternative concepts of JH action were created. The first is the well known and frequently

publicised theory of Gilbert-Riddiford, which claims that insect development is regulated by a moulting hormone (ecdysteroid) from the prothoracic glands (PG) released in response to prothoracicotropic hormone (PTTH) of the brain. The second hormone, juvenile hormone (JH), released from corpora allata, is assumed to be the sesquiterpenoid epoxy-homofarnesoate ester (JH-I), isolated from abdomens of the male Cecropia silkworms. The large, medium or small concentrations of JH-I are claimed to cause the respectively larval, pupal or adult development. There exist numerous reportst which claim that the hormonal activity of JH-I depends on enzymes (esterase) and genes (*Met* gene) of the peripheral tissue and organs[1].

The second, theory of Novák-Sláma proposes that PG do not regulate moults[1]. These peripheral glands are exclusive targets of the corpus allatum hormone (not PTTH) and their function depends on metabolic formation of water by total combustion of dietary lipids during larval growth[2]. The disintegrating PG release the polyhydroxylated sterol (ecdysone) for reutilisation of sterol nucleus during the non-feeding stages. Recent results show that, in contrast to 50-year old beliefs, JH-I is not the true corpus allatum hormone; it is a trivial excretory product of male exocrine accessory sexual glands (colleterial glands), which is translocated into females with ejaculate[3]. Larval development and metamorphosis are regulated by simple combinations of two hormones of the central neuro-endocrine system: a) neuropeptides produced in neuro-secretory cells of the brain (stimulation of moult cycles and ecdyses), and; b) corpus allatum hormone stimulating larval somatic growth and reproduction in adult stage. The role of these hormones is epigenetic, i. e. they control the functions of genes and enzymes in the subordinated peripheral target tissues according to the inherited instructions coded on the genome[1]

The first substances with JH activity (juvenoids) were acyclic or monocyclic sesquiterpenoids related to farnesol (excrements of the beetle *Tenebrio* and yeasts), or todomatuic acid ester juvabione (paper factor from the wood of balsam fir).

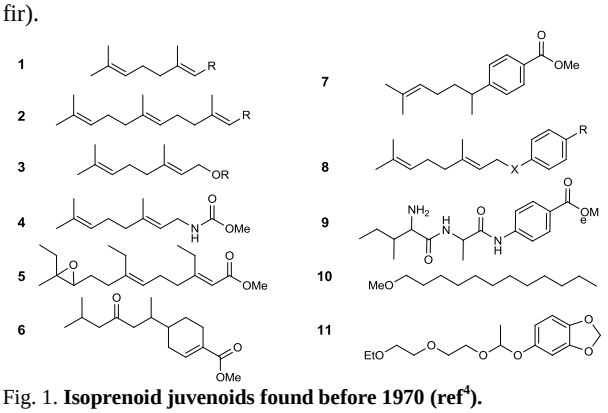

Fig. 1. **Isoprenoid juvenoids found before 1970 (ref[4]).**

General belief that juvenoids could be later used as a nontoxic substitution for insecticides, the research of JH attracted a vast number of academic and industrial institutions that published or patented hundreds of new isoprenoid analogues of JH every year (review[5]). After 1980, the number of terpenoid or nonterpenic juvenoids was estimated to more than 4000 synthetic compounds, some of which exhibited JH activity more than a million-fold higher in comparison with JH-I[6]. Certain juvenoids (methoprene, phenoxycarb, pyriproxyfen) were practically used in insect control.

Actually, the inhibitory effect of JH on insect metamorphosis is very simple. In contact of insect body with a juvenoid, the last larval instars do not develop into pupae or adults but usually make a giant supernumerary larval instar (see Figure 2).

Fig. 2. **From left: Normal larva, normal adult, JH-intermediate and JH-induced giant larva of *Dysdercus* (from[5]).**

Provided that the sesquiterpenoid JH-I as well as 4000 of the synthetic juvenoids cannot be the true corpus alla-tum hormone of insects[1], a question arises what is the real nature of JH hormone of insects. With respect to the striking evolutionary analogies between the corpus allatum of insects and adenohypophysis of vertebrates, there are certain indications that the true JH of the corpus allatum should be a peptide or protein.

REFERENCES

1. Sláma K.: Terr. Arthropod Rev. *6*, 257 (2013).
2. Sláma K., Lukáš J.: Eur. J. Entomol. *110*, 221 (2013).
3. Paroulek M., Sláma K.: Life: The Excitement of Biology *2*, 102 (2014).
4. Sláma K.: Ann. Rev. Biochem. *40*,1079 (1971).
5. Sláma K., Romaňuk M., Šorm F.: *Insect Hormones and Bioanalogues*,Springer Wien, New York, 477pp. (1974).
6. Sláma K., in: *Proc. 3rd Internat. Conf. Urban Pests*, Hronov 1999, Czech Republic, 9-25.

SYNTHESIS OF STEROIDS POSSESSING AN ASYMMETRIC CENTER AT C-25 ATOM

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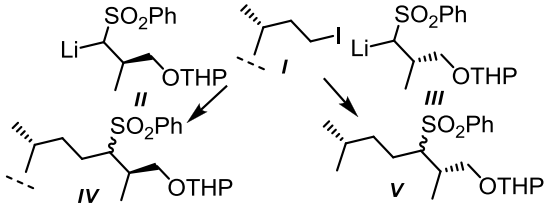
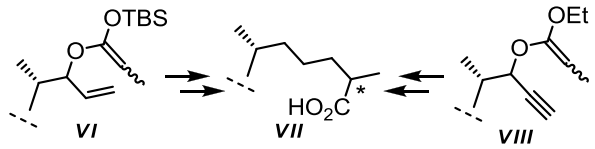
An asymmetric center at C-25 is a common feature of a number of natural steroids, including cholic acid biosynthetic intermediates, metabolites of brassinosteroids, and dafachronic acids. The formation of this structural element is still a challenge in syntheses of the above steroids. Three approaches have been suggested for a solution of the problem.

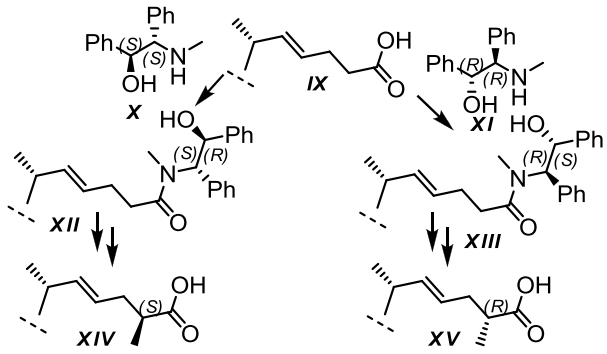
The first one relies on a convergent strategy and includes coupling of chiral synthones ***II*** and ***III*** with a steroidal fragment ***I***[1] as key step.



The second approach makes use of Ireland or Johnson orthoester variants of Claisen rearrangement of allylic or propargyl enol ethers ***VI*** and ***VIII***, respectively[2].



Amidation of ***IX*** with pseudoephenamines ***X*** and ***XI*** followed by methylation of the formed amides ***XII*** and ***XIII*** were used for the preparation of (25*S*)- and (25*R*)-acids ***XIV*** and ***XV***.



REFERENCES
1. Khripach V. A., Zhabinskii V. N., Konstantinova O. V., Khripach N. B., Antonchick A. V., Antonchick A. P., Schneider B.: Steroids *70*, 551 (2005).
2. Ermolovich Y. V., Zhabinskii V. N., Khripach V. A.: Steroids *78*, 683 (2013).

ORAL COMMUNICATIONS

DIMER SESQUITERPENE LACTONES; DISTRIBUTION IN NATURE AND SEARCH OF NEW COMPOUNDS

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Sesquiterpene lactones are the widest group among all natural isoprenoids by amount of compounds (currently, there are seven thousand compounds) found out in natural sources and by variety of structural types, i.e. structures of carbon skeleton of molecule.

Dimer sesquiterpene lactones are considered to be perspective in the group of natural terpenoids, which probably originated by biosynthesis with enzymes catalyzed with the Diels-Alder reaction. We have analyzed accessible literary data and have determined that amount of isolated dimer sesquiterpene lactones exceeds 240 compounds. It is well known the concepts about biomimetic synthesis of these complex natural metabolites and, therefore, full synthesis of their molecules and determination of the biogenetic transformations of dimer sesquiterpenoids is an a crucial task in the field of chemistry of natural isoprenoids.

In comparison with monomeric sesquiterpene lactones, dimer compounds have the limited distribution, for example, guaiane dimers are illustrative for plants of genus *Artemisia*, eremophilene - genus *Ligularia* and lindenane dimers are illustrative chemotaxonome feature for family *Chloranthus*.

In order to isolate dimer sesquiterpene lactones from plants of Kazakhstan's flora we are conducting the chemical studying plants of *Achillea*, *Artemisia*, *Ambrosia*, *Inula*, *Ligularia*, *Handelia*, *Pulicaria*, *Senecio*, *Tanacetopsis* genera.

Research results of antimicrobic, antivirus, anti-inflammatory and cytotoxic activities of extracts of separate species allow us to recommend *Artemisia absinthium* L., *Artemisia sieversiana* Willd., *Inula britannica* L., *Ligularia macrophylla* L. and *Handelia trichophylla* Schrenk. ex Fisch. et Mey as potential sources of biologically active dimer sesquiterpene lactones.

NEW TRITERPENIC DERIVATIVES WITH DIMERIC STRUCTURE

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In recent years, one of the topical directions of triterpene chemistry has become the synthesis of compounds that contain several carbocyclic backbones within their molecules. The combination in one molecule of two triterpenic particles results not only in the obtaining of new, interesting structures, but also in the appearance of new properties. The way of the formation of new dimeric derivatives is connected with the specific structure of mother triterpenic compound (*e.g.* oleanolic acid) and its chemical activity.

The triterpenic dimers comprise quite a unique class of compounds and really seldom occur in nature.

The synthetic triterpenic dimers are obtained only for some derivatives of lupane, oleanane and ursane groups.

The main aim of our interests was how to join two of the same or different triterpenic structures to form „dimers". The reactivity of hydroxyl, formyl, hydroxyimino or carboxyl functions were applied. The worked out methods of dimerization can be devided into four groups: 1) *via* C-3 modified or free COOH group, 2) *via* C-3 modified OH group, 3) *via* C-2 formyl group, 4) *via* C-3 hydroxyimino group. Some examples of the obtained dimers are presented in Figure 1.

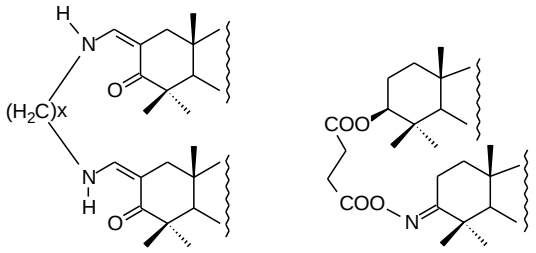

Fig. 1. **Different types of dimeric derivatives of triterpenes**

In order to obtain new dimeric derivatives, oleanolic acid was transformed into derivatives within A ring as well as COOH group with the use of reactions such as: transformation of COOH group into amide, benzyl or methyl ester, acylation, hydrolysis, Jones oxidation, oxime formation, formylation. The received compounds were subjected to dimerization with the use of alkyl dihalides, diamines, dicarboxylic acid anhydrides.

In our experiments methods of the synthesis of dimeric derivatives and the optimization of reaction conditions were worked out. The structures of the resulted compounds were elucidated on the basis of spectral data. New dimeric compounds will be subjected to biological tests *e.g.* to evaluate their anticancer activity and to find the relationships between their activity and structure. The X-ray analysis will be performed for the selected dimers in order to evalueate the mutual location of both parts of dimer.

TAXOL – 25 YEARS AFTER

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25 years ago taxol was a hot scientific topic. When reading the articles today, everybody believed that taxol is a magic bullet which can combat cancer worldwide and that the only problem is, to produce enough of this complicated natural product. And what is the situation now?

Taxol, or paclitaxel, what is now its generic name, is a standard drug and standard subject of pharmaceutical business. The annual world consumption is about 800 kg of the active substance and it is growing only very slowly. The substance is manufactured by three different procedures: isolation of natural paclitaxel either from natural biomass or from fermentation broth obtained by cultivation of plant cells or by partial synthesis from 10-deacetyl baccatin III or 9-dihydro-13-acetyl baccatin III.

Besides paclitaxel, some other taxanes are used as anticancer drugs. While docetaxel (Taxoter) is well known and its world market is comparable with that of paclitaxel, some others were approved recently (cabazitaxel) or are still under development.

REFERENCES

1. Hegi G., in: *Illustrerte Flora von Mitteleuropa*, p.1467. A. Pichler's Witwe & Sohn, Wien 1906.
2. Popović V., Heyerick A., Petrović S., Van Calenbergh S., Karalić I., Niketić M., Deforce D.: Phytochemistry *87*, 102 (2013).
3. Popović V., Heyerick A.,.Petrović S., Van Calenbergh S., Karalić I., Niketić M., Deforce D.: Rec. Nat. Prod. *7*, 245 (2013).
4. Merfort I.: Curr. Drug. Targets. *12*, 1560 (2011).
5. Dall'Acqua S., Linardi M. A., Maggi F., Nicoletti M., Petitto V., Innocenti G., Basso G., Viola G.: Bioorg. Med. Chem. *19*, 5876 (2011).

SYNTHESIS AND BIOACTIVITY OF 28-NORBRASSINOLIDE AND ITS BIOSYNTHETIC PRECURSORS

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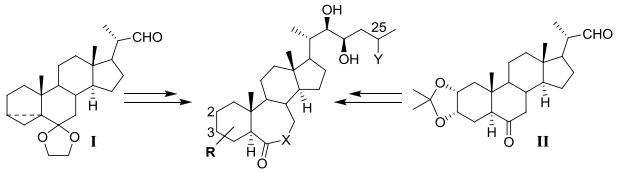
Since the discovery of brassinosteroids (BRs) in the late 1970s up to now a large amount of research on their chemical synthesis, biological activity, metabolism and biosynthesis has been conducted[1]. These data refer mostly to brassinolide and its 28C-derivatives. The same cannot be said for BRs of other carbon skeleton, specifically of cholestane.

In recent years it was revealed, that brassinosteroids as the typical phytohormones have an impact not only on plants, but also on living organisms outside the plant kingdom. Their ability to suppress growth and induce the

death of cancer cells in animals[2,3] is even more interesting. The fact that cholesterol is the main sterol of mammals and humans is an additional stimulus for a more detailed study of 27C-BRs.



III: 2α,3α-OH, X= O, Y= CH_3 **VII**: 3-keto, X= - , Y= CH_3
IV: 2α,3α-OH, X= - , Y= CH_3 **VIII**: 2α,3α-epoxy, X= - , Y= CH_3
V: 3β-OH, X= - , Y= CH_3 **IX**: 2α,3α-OH, X= - , Y= CD_3
VI: Δ^2, X= - , Y= CH_3 **X**: 2α,3α-OH, X= O, Y= CD_3

In this work we report a new efficient synthesis of 28-norbrassinolide **III**, its potential biosynthetic precursors **IV-VIII** and their trideuterated analogues from stigmasterol and 24-epicastasterone *via* corresponding intermediate aldehydes **I** and **II**. The biological activity of these compounds as the proliferation regulators will be shown.

REFERENCES
1. Khripach V., Zhabinskii V., de Groot A.: *Brassinosteroids. A new class of plant hormones*. Academic Press, San Diego 1999.
2. Malíková J., Swaczynová J., Kolá Z., Strnad M.: Phytochemistry *69*, 418 (2008).
3. Steigerová J., Oklestkova J., Levková M., Rárová L., Kolá Z., Strnad M.: Chem. Biol. Interact. *188*, 487 (2010).

SYNTHESIS AND *in vitro* PHARMACOLOGICAL EFFECTS OF NOVEL 17-OXADIAZOLYL ANDROSTENES

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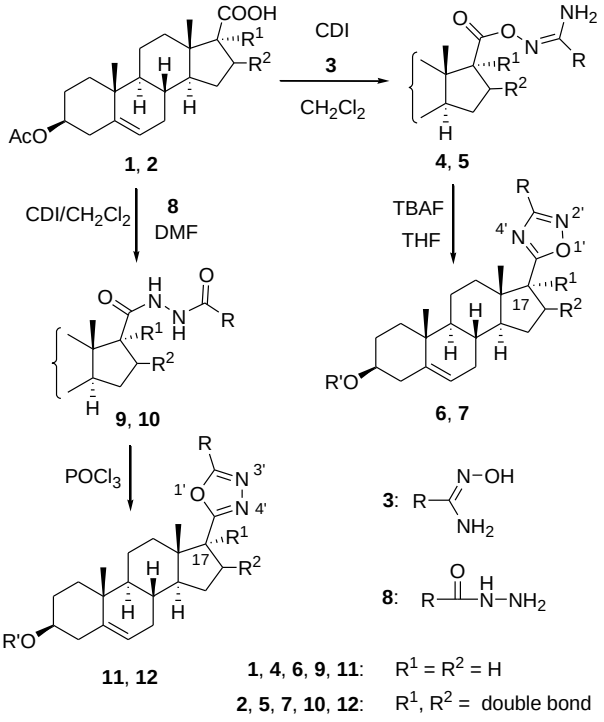
In recent years considerable attention has been devoted to the synthesis and pharmacological studies of 17-*exo*-heterocyclic steroids in view of their potential effectiveness in the treatment of androgen-dependent diseases by inhibiting 17α-hydroxylase-$C_{17,20}$-lyase (CYP17)[1].

Our goal was to introduce 1,2,4- and 1,3,4-oxadiazole moieties into the sterane core. CDI-Activated carboxylic acids **1** and **2** were reacted with different amidoximes (**3**) or acylhydrazines (**8**), respectively, to afford the corresponding intermediates (**4**, **5** or **9**, **10**), which were then subjected to cyclization to furnish **6**, **7** or **11**, **12** under appropriate conditions (Scheme 1)[2]. Some heterocyclic derivatives displayed significant *in vitro* inhibition of CYP17, and exerted marked cytotoxic activity against cell lines of diverse origin.



Scheme 1. **Synthesis of 1,2,4- and 1,3,4-oxadiazolyl androstenes**

REFERENCES
1. Salvador J. A. R., Pinto R. M. A., Silvestre S. M.: J. Steroid Biochem. Mol. Biol. *137*, 199 (2013).
2. Kovács D., Wölfling J., Szabó N., Szécsi M., Kovács I., Zupkó I., Frank É.: Eur J. Med. Chem. *70*, 649 (2013).

TOTAL SYNTHESIS OF PANDAROSIDES E-J

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Pandarosides are steroidal saponins identified as constituents of the Caribbean marine sponge *Pandaros acanthifolium* extract. The major fraction from the extract contained pandarosides A-D and their methyl esters. Reinvestigation of the chemical constitution of the extract in search for more bioactive constituents resulted in the isolation of six new steroidal glycosides named pandarosides E-J, as well as their methyl esters. All of this metabolites share an unusual oxidized (2-hydroxycyclopent-2-enone) D-ring with a β-configuration at C-14. Differences with the formerly isolated pandarosides A-D lay in the B- and C-rings, in the alkyl chain, and in the sugar section, where for the first time for this family rhamnose and xylose structures were identified (Figure 1). Only a few of these saponins have been proven to exhibit antiprotozoal bioactivity. The majority of those metabolites showed an *in vitro* activity

against several parasitic protozoa. Especially active was pandaroside G, which strongly inhibited the growth of *Leishmania donovani* and *Trypanosoma brucei rhodesiense* (responsible for tropical diseases)[1].

Now, we present the synthesis of disaccharide and aglycone parts of the target structures. Initially prepared monosaccharides have been coupled to deliver: Glc-β-1,2-GlcA, Xyl-β-1,3-GlcA, Rha-α-1,4-GlcA glycoside parts of three pandaroside units H, I, J. Further glycosylation with cholesterol molecule have been done as a model study. Steroidal skeleton was obtained starting from stigmasterol in 21 steps.

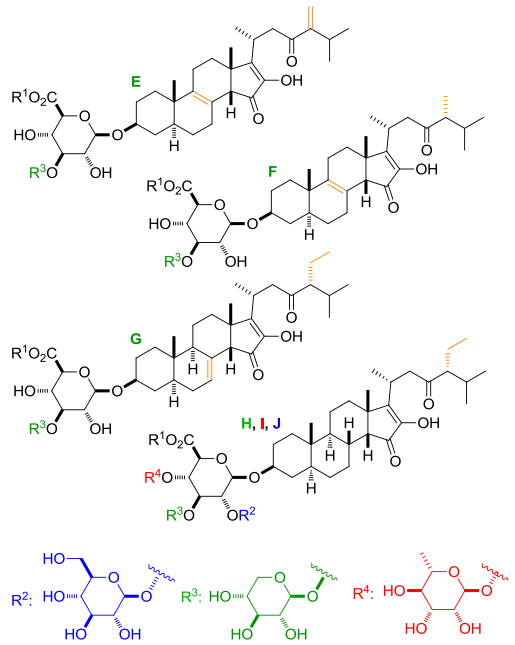

Fig. 1. **Pandarosides E-J**

REFERENCES

1. Regalado E. L., Tasdemir D., Kaiser M., Cachet N., Amade P., Thomas O. P.: J. Nat. Prod. *73*, 1404 (2010).

REGULATION OF PRIMARY PHOTOSYNTHETIC PROCESSES IN PLANTS BY BRASSINOSTEROIDS AND ECDYSTEROIDS

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Ecdysteroids (ECs) and brassinosteroids (BRs) are two groups of plant steroids that are somewhat similar but differ in details of their structures as well as in other aspects (biosynthesis, levels, distribution in plants *etc.*). BRs are involved in positive regulation of many plant processes including photosynthesis[1]. However, it is still not clear what exactly causes BR-caused improvements of photosynthetic efficiency and particularly the possible role of BRs in the regulation of primary photosynthetic processes deserves further examination. ECs´ function in plants is mostly unknown and there are some indications that they could be involved not only in plant defense against phytophages but in regulation of other plant processes (including photosynthesis) as well[2,3].

We examined the effects of exogenous application of 24-epibrassinolide [(22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-7-oxa-7α-homo-5α-cholestan-6-one; ***I***] and 20-hydroxyecdysone (2β,3β,14α,20R,22R,25-hexahydroxy--5β-cholest-7-en-6-one; ***II***) on various parts of photosynthetic electron-transport chain using chlorophyll fluorescence kinetics analysis (OJIP transient)[4]. Various concentrations (10^{-4} to 10^{-14} M) of ***I*** and ***II*** aqueous solutions were used, several plant species (*Zea mays* L., *Spinacia oleracea* L., *Vicia faba* L., *Pisum sativum* L., *Brassica napus* L., *Raphanus sativus* L.) were compared and the response of photosynthetic apparatus in thylakoid membranes was assessed 1, 5, 24, 48, 72 and 168 hours after steroid application. We found that (i) various plant species respond to ***I*** or ***II*** application differently; (ii) the effect of steroids on primary photochemistry can be both positive and negative depending on their concentration, plant species and time elapsed from their application; (iii) different components of photosynthetic electron-transport chain (*e.g.* Photosystems 1 and 2) can have different response to steroids; (iv) exogenously applied ECs can affect primary photosynthetic processes at least in some plant species in concentrations similar to those that are effective for BRs.

This work was supported by the Czech Science Foundation (grant 501/11/1650) and the Grant Agency of Charles University in Prague (grants B/BIO/612612 and SVV-2014-260081).

REFERENCES

1. Holá D., in: *Brassinosteroids: A class of plant hormone*, Chapter 6, pp. 143. Springer Science + Business Media B.V., Dordrecht-Heidelberg-London-New York 2011.
2. Golovatskaya I.F.: Russ. J. Plant. Physiol. *51*, 452 (2004).
3. Bajguz A., Koronka A.: Plant Physiol. Biochem. *39*, 707 (2001).
4. Stirbet A., Govindjee: J. Photochem. Photobiol. B: Biol. *104*, 236 (2011).

SYNTHESIS OF LONG-TERM DEHYDROCHLOROMETHYLTESTOSTERONE METABOLITES

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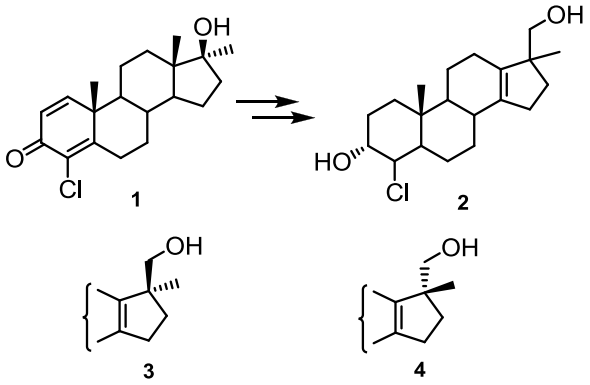
The long-term detection of anabolic steroid metabolites is essential for the antidoping analysis. A number of recently identified biotransformation products (e.g. **2**) proved to be of a great value for dealing with dehydrochloromethyltestosterone (**1**) abuse as these metabolites could be detected over an extended period of time[1].

In the present report, the synthesis of compounds **2** will be discussed. The main attention is focussed on the development of an efficient approach to the formation of 18-nor-17-hydroxymethyl-17-methyl fragments **3** and **4**.



REFERENCE
1. Sobolevsky T., Rodchenkov G.: J. Steroid Biochem. Mol. Biol. *128*, 121 (2012).

PHOTOCHEMICAL ISOMERIZATION OF 23-OXOSAPOGENINS

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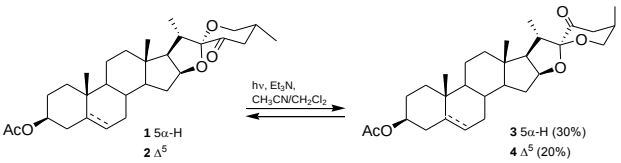
Spirostane saponins are glycosides widely distributed in the plant kingdom with various biological activities[1]. Spirostan sapogenins were used in the synthesis of furostanic and spirostanic analogues of brassinosteroids, OSW-1 aglycone, cephalostatin derivatives, anticancer sapogenins[2].

Naturally occurred spirostane sapogenins with a characteristic spiroketal system, have an *R* configuration at the spiro carbon atom (C22). The molecular modeling (MM+) calculations showed that normal sapogenins (22*R*) have lower steric energy that their epimers at C22[3].

It is well known that acid media promote isomerization of spirostanic sapogenins. However none of the known isomerization methods allow to change configuration at the spiro carbon atom without alteration of configuration at other side chain chiral centers[4].

It was observed that during irradiation of 23-oxospirostanes occurs isomerization at the spiro carbon atom [Scheme 1].[5] The spectroscopic and X-ray analysis unequivocally proved the structure of products. The results will be presented.



Scheme 1. **Photoinduced isomerization of 23-oxosapogenins**

REFERENCES
1. a) Hostettmann K.; Marston A.: *Saponins*; University Press: Cambridge, UK 1995; b) Sautour M.; Mitaine-Offer A.-C.; Lacaille-Dubois M.-A.: J. Nat. Med. *61*, 91 (2007)
2. Gryszkiewicz-Wojtkielewicz A., Jastrzębska I., Morzycki J.W., Romanowska D.B.: Curr. Org. Chem. *7*, 1257 (2003).
3. Iglesias-Arteaga M. A., Jastrzębska I., Morzycki J.W.: Polish J. Chem. *80*, 667 (2006).
4. Jastrzebska I.: Curr. Org. Chem. *16*, 353 (2012).
5. Jastrzębska I., Górecki M., Frelek J., Santillan R., Siergiejczyk L., Morzycki J.W.: J. Org. Chem. *77*, 11257 (2012).

ANTIHORMONAL POTENTIAL OF SELECTED D-HOMO AND D-SECO ESTRATRIENE DERIVATIVES

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Since many estrogen derivatives exhibit anti-hormone or enzyme inhibition potential, a large number of steroidal derivatives have been synthesized from appropriate

precursors, in order to obtain potential therapeutics for the treatment of hormone-dependent cancers.

In molecular docking studies, selected D-homo and D-seco estratriene derivatives were predicted to bind strongly to estrogen receptor α (ERα), aromatase and lyase, suggesting they could be good starting compounds for antihormonal studies.

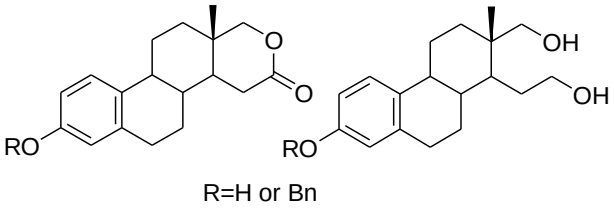

Fig. 1. **Chemical structures of potential antihormones**

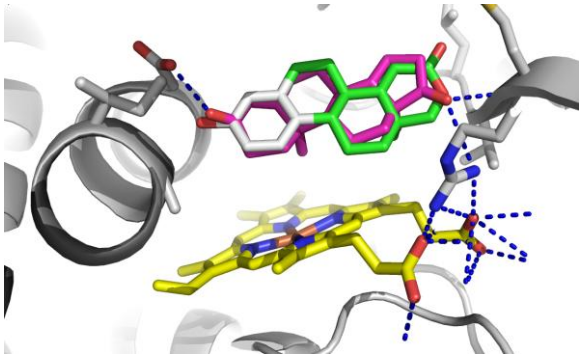

Fig. 2. **Aromatase docked with 3-hydroxy D-lactone; docking energy: -10.39 kcal/mol. Note D-ring lactone forms hydrogen bonds just like androstenedione (magenta)**

Test results *in vivo* suggest these compounds do not possess estrogenic activity, although some showed weak anti-estrogenic properties. *In vitro* anti-aromatase and anti-lyase assays showed partial inhibition of these two enzymes, while some compounds activated aromatase. Aromatase activators are capable of promoting estrogen synthesis for treatment of pathological conditions caused by estrogen depletion.

Research was funded from the projects 114-452-3716/2011-01 and 114-451-3600/2013-03.

NEUROSTEROID NMDA RECEPTOR INHIBITORS: CURRENT INSIGHT INTO OUR STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

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Our interest in the synthesis of *N*-methyl-D-aspartate receptor (NMDAr) inhibitors ideally with neuroprotective effect is based on common knowledge that the activation of NMDA receptor, which belongs to the family of glutamate activated receptor, is essential for synaptic plasticity, learning, and memory. However, overexcitation of the NMDAr can also induce cell death. This excitotoxicity is often seen in conjunction with ischemia injuries in the brain, and is thought to contribute to the neurodegeneration associated with various forms of dementia. Of our interest is the fact that progesterone and its reduced metabolites also behave as endogenous neuroprotectives. In particular, 3α,5β-pregnanolone sulfate has been shown to be an effective, use-dependent antagonist of the NMDAR with a high neuroprotective potential. However, sulfate moiety can be easily cleaved by enzymes in the organism. Therefore, we have synthesized and examined series of compounds bearing substituents at C-3 with negatively and positively charged substituents, as well as zwitterions. These C-3 substituents differ by a type and length and new structure-activity relationships have been established. Also, we have introduced various substituents to different position of steroid skeleton (e.g. C-7, C-17, C-20) as well as modifications in the D-ring of steroid skeleton. Subsequently, the patch-clamp and imagining recordings from HEK293 cells expressing NR1/NR2B receptors and cultured rat hippocampal neurons were used to establish the NMDAR inhibition and IC_{50} values. Moreover, our *in vivo* experiments show that these neurosteroid ligands are able to cross the blood brain barrier and do not induce psychotomimetic symptoms (such as hyperlocomotion and sensorimotor gating deficit). These findings provide a possible new therapeutic approach for the treatment of diseases induced by NMDA receptor overactivation. The structure-activity relationship, detailed synthesis, and relationship of structure vs. IC_{50} values will be discussed.

Supported by grant TE01020028 Center for Development of Original Drugs from the Technology Agency of the Czech Republic, grant 303/12/1465 from the Grant Agency of the Czech Republic, and RVO 61388963.

HOW DOES IT WORK? KINETIC INSIGHTS INTO THE THERMALLY INDUCED REARRANGEMENT OF PINAN-2-OL

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Rearrangement reactions of terpenoids play a major role in industrial synthesis of fine chemicals. For example the thermal conversion of pinan-2-ol (***I***) leads to linalool (***II***). Like many other terpenoids ***II*** has a characteristic odor

which allows application as fragrance in consumer products. Furthermore, they serve as starting material for the synthesis of chiral ligands or auxiliaries, as building blocks for vitamins and other fine chemicals and in the pharmaceutical industry[1].

Within the present study, the thermal rearrangements of ***I*** and ***II*** were investigated under identical experimental conditions using a flow-type reactor made of quartz glass with residence times between 0.4-2.2 s^{-1} and in a temperature range of 350-600 °C.

Small variation of reaction conditions such as reaction temperature, residence time or reactor geometry can change the product range and influence the global reaction rate and linalool selectivity. Experiments revealed that besides ***II*** the rearrangement of ***I*** leads to the formation of side-reaction products β-terpineol (***III***), myrcene (***VII***), and limonene (***IX***). Furthermore consecutive reaction of ***II*** yielded cyclopentanol derivatives (***V*a-d**)[2].

On the basis of pyrolysis experiments rate constants and kinetic parameters (frequency factors $\log_{10}A$, activation energies E_a, enthalpies $\Delta H^{\neq}$ and entropies $\Delta S^{\neq}$) were calculated, using a model of competitive first-order parallel and consecutive reactions to describe the reaction network. Furthermore, the mechanisms for the thermal isomerization of ***I*** and ***II*** are discussed.

Additionally, different kinetic behavior is investigated when performing several experiments using different surface-to-volume-ratios or adding some liquid additives in the feed stream to manipulate the amount of side products formed in this reaction.

REFERENCES
1. a) Swift K. A. D.: Top. Catal. *27*, 143 (2004); b) Corma A., Iborraa S., Velty A.: Chem. Rev. *107*, 2411 (2007); c) Stolle A., Ondruschka B., Hopf H.: Helv. Chim. Acta *92*, 1673 (2009).
2. Leiner J., Stolle A., Ondruschka B., Netscher T., Bonrath W.: Molecules *18*, 8358 (2013).

ECDYSTEROIDS NEW ROLE – EFFECTORS OF PHOTOSYNTHETIC ENZYMES (O_2 PRODUCTION AND CO_2 BINDING)

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Our results show that ecdysteroids are able to increase the yield of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) – mediated reaction in which CO_2 is fixed into organic matter[1], and influence the water cleavage reaction evolving oxygen[2]. By affinity chromatography of plant extracts of different plant species on a set of columns with immobilised oxysterols we identified a range of proteins able to bind ligands like 24-epibrassinolide or 20-hydroxyecdysone[3]. The ability to bind oxysterols found in enzymes involved in photosynthesis led us to examine the effect of such compounds on the biological activity of the involved proteins. Studies were performed on different models using both *in vivo* and *in vitro* approaches (purified enzymes, chloroplasts, leaf discs). Using radioactively labelled CO_2 an increase of its sequestration by RuBisCO was proved as result of 20-hydroxyecdysone treatment[1]. Effect of its application on various photosynthetic parameters was followed on whole plants of spinach and maize[4].

Ecdysteroids as insect hormones have been investigated thoroughly but knowledge of their function and the mechanism of action in plants and other organisms is not so detailed. As far as we know, these results are the first to suggest a new potential biological function of phytoecdysteroids — regulation of photosynthesis.

The financial support by GACR project No. 501/11/1650 is greatly appreciated.

REFERENCES
1. Uhlik O., Kamlar M., Kohout L., Jezek R., Harmatha J., Macek T.: Steroids *73*,1433 (2008).
2. Rothova O., Hola D., Kocova M., Tumova L., Hnilicka F., Hnilickova H., Kamlar M., Macek T.: Steroids *85*, 44 (2014).
3. Kamlar M., Uhlik O., Kohout L., Sanda M., Macek T.: J. Biotechnol. *150*, S490 (2010).
4. Hola D., Kocova M., Rothova O., Tumova L., Kamlar M., Macek T.: Acta Physiol. Plant. *35*, 3489 (2013).

CAROTENOIDS SYNTHESIS IN NICOTIANA TRICHOMES

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Carotenoids are a broad group of C_{40} isoprenoids, which includes carotenes and their derived oxygenated called xanthophylls, with many functions, not only in the bacteria, plants, and fungi that synthesize them, but in the animals that acquire them in their diet[1]. Carotenoid accumulation in plants has been manipulated by metabolic engineering resulting in increased levels in seeds, leaves, and fruits[2]. Glandular trichomes are protuberances encountered at the surface of many plants. These structures possess metabolically active cells which synthesize specific compounds. In tobacco (*Nicotiana tabacum*), these cells produce diterpenes and therefore geranylgeranyl diphosphate, which is also the precursor of carotenoids[3]. However, to the best of our know-

ledge, no attempt of carotenoids engineering in trichomes has been reported yet. Our aim is explore the capacity of glandular trichomes of *N. tabacum* for carotenoids synthesis.

We have prepared constructions[4] carrying two genes (*carRA* and *carB*) required for β-carotene synthesis in fungi. These constructions have been inserted in *N. tabacum* via *Agrobacterium tumefaciens*. Plastid targeting sequences were added to the coding sequences and their expression was directed by trichome specific promoters[5]. We have regenerated several transgenic lines whose trichomes show the typical colors of carotenoids. Progress in the characterization of these lines will be presented. Our results demonstrate the capacity of trichomes for the biosynthesis of these tetraterpenoids and offer perspectives for the engineering of carotenoid-derived compounds.

REFERENCES
1. Álvarez R., Vaz B., Gronemeyer H., R. de Lera A.: Chem. Rev. *2014*, 144.
2. Ye V.M., Bathia S.K.: Food Sci. Biotechnol. *2012*, 21.
3. Tissier A., Sallaud C., Rontein D., in: *Isoprenoid Synthesis in Plants and Microorganisms, New Concept and Experimental Approaches*. Chapter. 18. Springer, New York 2013.
4. Werner S., Engler C., Weber E., Gruetzner R., Marillonnet S.: Bioeng. Bugs 2012, 3.
5. Tissier A. in: *Transgenic Plants: Advances and Limitations*. InTech, DOI: 10.5772/32101. Croatia, 2012.

SULFUR-CONTAINING DERIVATIVES OF MONO- AND BICYCLIC NATURAL MONOTERPENOIDS: SYNTHESIS, STRUCTURE, BIOLOGICAL ACTIVITY

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Thiylation of natural mono- and bicyclic monoterpenoids and the latest transformation pathways of the newly formed sulfur-containing derivatives were investigated[1,2].

Using the data of X-ray crystallography, IR spectroscopy, and quantum-chemical calculations, it was established that for the diastereomeric pinanyl sulfoxides, there is a tendency to "racemic compound-like" type dimerization in crystals, melts, and solutions[3]. For the first time, the stereochemical transformation accompanying the crystallization of homochiral pinanyl sulfone, is found and studied. We suggest to use a new term – "crystallization-induced diastereomerization" – to designate this phenomenon[4].

The formation of stable dimer associates is extremely interesting with respect to potential bioactive properties of this compound, since it is known that the latter greatly depend on the type of intramolecular associates in solution. Hemocoagulating activity of thioterpenoids of human blood plasma *in vitro* were studied. The most water-soluble sulfoxide inhibits completely the spontaneous and induced by collagen and arachidonic acid aggregation of platelets; it also reduces the coagulating activity of human blood plasma.

REFERENCES
1. Ishmuratov G. Yu., Yakovleva M. P., Tukhvatshin V. S., Talipov R. F., Nikitina L. E., Artemova N. P., Startseva V. A., Tolstikov A. G.: Chem. Nat. Compd. *50*, 22 (2014).
2. Nikitina L. E., Artemova N. P., Startseva V. A.: *Natural and Thio-modified Monoterpenoids* [in Russian]. Lap Lambert, Saarbrücken 2012.
3. Startseva V. A., Nikitina L. E., Lodochnikova O. A., Klimovitskii A. E., Aref'ev A. V., Artemova N. P., Bodrov A. V., Musin R. Z., Klimovitskii E. N.: Phosphorus, Sulfur, Silicon, Relat. Elem. *189*, 615 (2014).
4. Lodochnikova O. A., Startseva V. A., Nikitina L. E., Bodrov A. V., Klimovitskii A. E., Klimovitskii E. N., Litvinov I. A.: CrystEngComm *16*, 4314 (2014).

SYNTHESIS AND CHARACTERIZATION OF NEW STEROIDAL HETEROCYCLIC COMPOUNDS

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The search for steroid compounds analogs with improved biological properties includes, among others, chemical transformations of naturally occurring steroids into D-homo and D-seco steroidal compounds, which are an important group of modified steroids, exhibiting a variety of different biological activities[1,2]. In the development of new steroidal compounds with biomedical potential, we synthesized some D-seco and D-homo steroidal heterocyclic compounds in androstane and estrane series. One of them is 3β-hydroxy-16,17a-bis(oximino)-17-aza-D-homo-androst-5-ene, synthesized in several synthetic steps starting from dehydroepiandrosterone.

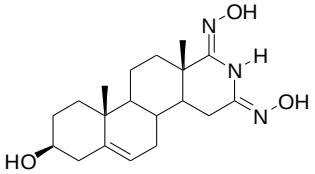

Fig. 1. **Chemical structure of 3β-hydroxy-16,17a-bis(oximino)-17-aza-D-homo-androst-5-ene**

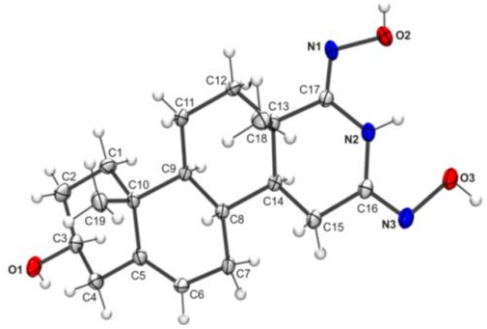

Fig. 2. **X-ray crystal structure of 3β-hydroxy-16,17a-bis(oximino)-17-aza-D-homo-androst-5-ene**

Research was funded from the projects 114-452-3716/2011-01 and 114-451-3600/2013-03.

REFERENCES

1. Penov-Gaši K. M., Oklješa A. M., Petri E. T., Ćelić A. S., Djurendić E. A., Klisurić O. R., Csanadi J. J., Batta G., Nikolić A. R., Jakimov D. S., Sakač M. N.: Med. Chem. Commun. *4*, 317 (2013).
2. Oklješa A. M., Jovanović-Šanta S. S., Klisurić O. R., Sakač M. N., Djurendić E. A., Jakimov D. S., Aleksić L. D., Penov Gaši K. M.: J. Braz. Chem. Soc. *24*, 1613 (2013).

VOLATILE ANALYSIS BY COMBINING GAS CHROMATOGRAPHY WITH NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Gas chromatography coupled with mass spectrometry (GC-MS) is the most commonly used method to analyze ecologically relevant or otherwise interesting volatiles such as terpenoids, fatty acid catabolites and aromatics from plants, insects or other organisms[1]. Since the introduction of GC-MS, comprehensive libraries have been created which make use of characteristic fragmentation patterns after electron impact ionization (EI). Together with retention indices, MS-library screening allows for the quick identification of known volatile compounds. Unknown compounds, however, often withstand their elucidation since an analysis solely based on fragmentation behaviour, molecular mass and retention time lacks the most important chemical information: the connectivity of the individual atoms comprising the compound.

NMR as the most powerful spectroscopic method is the method of choice for the analysis of organic compounds since it readily provides the atom connectivity information required for de novo structure elucidation. However, the occurrence of volatiles in complex blends is challenging for the application of NMR to elucidate such structures in a mixture. Online coupling of GC to NMR would be desired but is still on an experimental stage[2]. Therefore we used an offline combination of GC with NMR to separate and identify individual volatile components from plants and from plant enzyme assays. The analysis of such mass-limited mixtures required the use of cryogenic NMR probes and miniaturization of the NMR samples.

Here we present examples for the successful off-line combination of GC with NMR. Using a preparative GC-fraction collector for separation followed by cryoprobe NMR for detection, the structures of unknown compounds have been successfully identified.

REFERENCES

1. Baldwin I. T.: Curr. Biol. *20*, 392 (2010).
2. Gökay O., Albert K.: Anal. Bioanal. Chem. *402*, 647 (2012).

SESQUITERPENES FROM SELECTED *Laserpitium* L. SPECIES AS ANTIMICROBIAL AGENTS

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Species of the genus *Laserpitium* L. (Apiaceae) are medicinal plants used in European traditional medicine to treat disorders connected to inflammation and infection[1]. Previous investigation of this genus showed that *Laserpitium* species are particularly rich in sesquiterpene lactones and daucane esters[2,3]. Sesquiterpene lactones are pharmacologically active plant secondary metabolites, mainly investigated for their anti-inflammatory and antitumor properties[4]. Daucane esters are gaining scientific interest for their pro-apoptotic and antiproliferative activity[5]. Isomontanolide, guaianolide lactone isolated from *L. siler* roots, showed antimycobacterial activity[6]. The aim of the study was to test isolated compounds - sesquiterpene lactones and daucane esters, from three selected *Laserpitium* species for their antimicrobial properties, using microdilution method and monitoring inhibition of a bio-film formation. The aim of the study is to discover novel agents that may be

of a pharmaceutical importance, taken into account growing resistence of microorganisms.

Authors are grateful to European Commission Erasmus Mundus Action 2 Program for issuing a Basileus grant to Dr. Višnja Popović.

REFERENCES

1. Hegi G., in: *Illustrerte Flora von Mitteleuropa*, p.1467. A. Pichler's Witwe & Sohn, Wien 1906.
2. Popović V., Heyerick A.,.Petrović S., Van Calenbergh S., Karalić I., Niketić M., Deforce D.: Phytochemistry *87*, 102 (2013).
3. Popović V., Heyerick A.,.Petrović S., Van Calenbergh S., Karalić I., Niketić M., Deforce D.: Rec. Nat. Prod. *7*, 245 (2013).
4. Merfort I.: Curr. Drug. Targets. *12*, 1560 (2011).
5. Dall'Acqua S., Linardi M. A., Maggi F., Nicoletti M., Petitto V., Innocenti G., Basso G., Viola G.: Bioorg. Med. Chem. *19*, 5876 (2011).
6. Lechner D., Gibbons S., Bucar F.: Sci. Pharm. *74*, S94 (2006).

EXAMINATION OF ASSOCIATION-MICELLISATION OF NOVEL ETHYLIDENE DERIVATIVES OF BILE ACID

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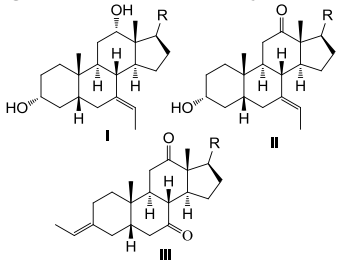
Bile acid salts (BS) are surfactants with steroid skeleton. They belong to group of planar surface active substances. Convex side of the steroid skeleton is hydrophobic while the concave side is hydrophilic[1,2]. In water solution BA form relatively small micelles with aggregation numbers from 2 to 13, whose building units mutually bind over their β (hydrophobic) side of the steroid skeleton[3]. In some BS micelles between building units hydrogen bonds are possible[3]. Micellar systems of BA are not only important for solubilisation of cholesterol [4], but they interact with certain drugs changing thus their bioavailability[5].



Scheme 1. **Tested bile acids' ethylidene derivatives (R = $CH(CH_3)CH_2CH_2COOH$)**

Since BS micelles are relatively small the aim is to increase their hydrophobic domain so in this paper micellisation of ethylidene BS derivatives ***I***, ***II*** and ***III*** (Scheme 1) is examined.

Table 1,
Parameters of micellisation: critical micelle concentration (CMC), aggregation number (*n*) and retention parameter of the reversed phase liquid chromatography (*k*)

BS	*k*	CMC [mM]	*n*
I	5.33	3.00	14.2
II	4.07	8.25	7.0
III	1.09	43.50	4.4

If BS derivatives with ethylidene group (Table 1) are compared to BS derivatives with α oriented OH group[6] instead of ethylidene group, it can be concluded that hydrophobicity of steroid skeleton is increased in a greater extend if ethylidene group is introduced in C_7 position than if it is in C_3 position. For derivatives ***I***, ***II*** and ***III*** hydrophobicity also determines CMC and *n*. However, for derivative ***I,*** if aggregation numer would be determined only with hydrophobicity, than *n* would have the value up to 10. This suggests that for derivative ***I*** hydrogen bonds are also possible.

This study was supported by the Ministry of Science and Technological Development of the Republic of Serbia (Project No. 172021). The work was also financially supported by the Provincial Secretariat for Science and Technological Development, AP Vojvodina, Republic of Serbia, Grant No. 114-451-2113/2014.

REFERENCES

1. Poša M., Kuhajda K.: Steroids *75*, 424 (2010).
2. Poša M.: Biochimie *97*, 24 (2014).
3. Poša M., Sebenji A.: Biochim. Biophys. Acta *1840*, 1072 (2014).
4. Poša M., Farkaš Z.: Collect. Czech. Chem. Commun. *75*, 767 (2010).
5. Poša M., Csanádi J., Kövér K., Guzsvány V., Batta G.: Colloids Surf. B *94*, 317 (2012).
6. Poša M.: Steroids *76*, 85 (2011).

EXEMESTANE METABOLITES: SYNTHESIS, STEREOCHEMICAL ELUCIDATION AND MECHANISM OF ACTION IN BREAST CANCER

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Exemestane (Aromasin®) is the only steroidal aromatase inhibitor orally active, long-lasting and safe for the treatment of hormone-responsive breast cancer in postmenopausal women. *In vivo*, it is transformed in several metabolites, being some of them active. Due to the huge clinical value of exemestane, we were interested in synthesizing epoxide derivatives, since they are proposed as potential metabolites and because it is known that, similarly to the double bonds, epoxide functions also allow the molecule to maintain planarity, which is an important feature required for the anti-aromatase activity[1]. Therefore, we have prepared epoxide derivatives (6β-spirooxiranandrosta-1,4--diene-3,17-dione ***I*** and 1α,2α-epoxy-6-methylenandrost-4--ene-3,17-dione ***II***), whose stereochemistry was unequivocally elucidated for the first time on the basis of NOESY experiments. Besides this, we were also interested in study two other well-established metabolites, 17β-hydroxy-6-methylenandrosta-1,4-dien-3-one ***III*** and 6-(hydroxymethyl) androsta-1,4,6-triene-3,17-dione ***IV***. The aromatase inhibitory activity was evaluated in microsomes and in MCF-7aro cells, a hormone-dependent breast cancer cell line. The antiproliferative effects of the compounds were also evaluated in this cell line. The two potential epoxide metabolites (***I*** and ***II***) revealed to be less potent aromatase inhibitors than exemestane in microsomes however, in cells, ***I*** exceeded the activity of exemestane. Metabolite ***III*** also showed higher activity in cells than exemestane. All the studied metabolites reduced cell viability in a more pronounced way than exemestane. Further, ***III*** reduced cell viability even in presence of estradiol, showing that other mechanisms than aromatase inhibition, are involved in their action. Ultimately, the exemestane activity can be due not only to itself, but also to their metabolites, through aromatase inhibition and/or other mechanisms.

Authors are grateful to FCT (Fundação para a Ciência e Tecnologia) for the strategic project PEst-OE/SAU/UI0177/2011 and for the project (PTDC/QUI-BIQ/120319/2010).

REFERENCE

1. Varela C., Tavares da Silva E., Amaral C., Correia da Silva G., Baptista T., Alcaro S., Costa G., Carvalho R. A., Teixeira N. A. A., Roleira F. M. F.: J. Med. Chem. *55*, 3992 (2012).

USE OF TERPENOIDS IN DESIGN OF NEW MEDICAL DRUGS

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Today synthetic transformations of natural compounds, including terpenoids - one of leading trends of creation of new medicinal drugs. More than 60 % of medicinal drugs used now in world medical practice are based on natural substances.[1]

We synthesized from triterpenoids Glycyrrhetinic acid and Betulin new compounds (I and II accordingly) with high anti-cancer activity[2].

Interaction of some monoterpenoids with set of aldehydes lead to the agents possessing analgesic properties.

Using camphor as a starting molecule we obtained agents with anti-flu (III) and myorelaxant (IV) activities.

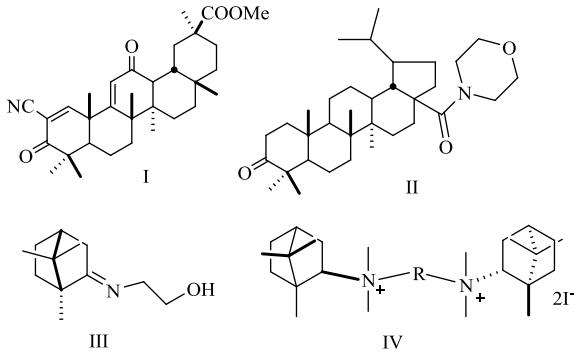

Fig. 1. **Structure of compounds I-IV**

The author is grateful to the Russian Foundation for Basic Research for financial support (grant N 13-03-00206a).

REFERENCES

1. Newman D. J., Cragg G. M.: J. Nat. Prod. *75*, 311 (2012).
2. Logashenko E. B., Salomatina O. V., Markov A. V., Korchagina D. V., Tolstikov G. A., Vlassov V.V., Zenkova M.A., Salakhutdinov N. F.: ChemBioChem *12*, 784 (2011).

IMMUNOASSAY OF BRASSINOSTEROIDS

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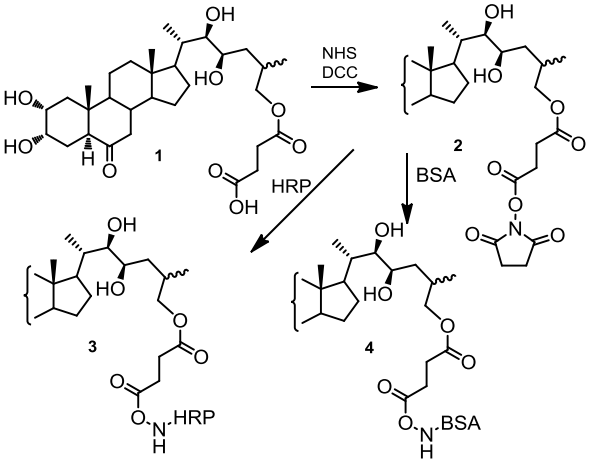
Brassinosteroids (BS) are low molecular bioregulators playing an important role in plant growth and development. Content of natural BS in plant sources is very low (lesser than 10^{-5} %). Based on ours and others' experiences in BS studying, immunoenzymatic assay could be considered as a method of choice for their routine analysis.

The (22*R*,23*R*)-2α,3α,22,23,26-pentahydroxy-5α-cho-le-stan-6-one 26-hemisuccinate **1** was used as a starting material for the preparation of conjugates with proteins. The conjugate **3** of 28-norcastasterone with BSA was used as immunogen for producing an antiserum specific for 6-keto-BS. The reaction of the ester **2** with a HRP gave the

conjugate **4**, which was used as a labeled antigen. The newly developed analytical system (**IA1**) is highly specific towards 6-keto-BS.



Apart from the elaborated in this work immunoassay system **IA1**, two other systems (**IA2** and **IA3**) were used for analysis of individual hormones of 28-homoBS series in plant material. The antibodies of the test system **IA2** specifically bind to steroids containing 2α,3α,22*R*,23*R*-tetraol and 7-membered 7-oxalactone B-ring (B-lactone-BS), and they have low (2-8 %) cross-reactivity with BS of 6-keto--series. The system **IA3** was designed to measure steroidal compounds bearing 2α,3α,22R,23R-tetraol and 24S-ethyl groups. It has low cross-reactivity with BS of 24*S*- and 24*R*--methyl series, but shows 100 % cross reactivity for 28-homobrassinolide and 28-homocastasterone.

Combined application of these three immunoassays allowed selective measuring the hormones in plant material. The correctness of the results was confirmed by independent chromatographic purification and HPLC-MS analysis.

This work was supported by Grant № X14P-139 of the Belarusian Republican Foundation for Fundamental Research.

WHAT ARE ECDYSTEROIDS: INSECT HORMONES, ESSENTIAL MAMMALIAN D-VITAMINS OR POLAR STEROLS USED FOR GROWTH IN PLANTS?

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The polyhydroxylated derivatives of 6-keto,7-dehydrocholesterol (Ecdysteroids; ECD) are distributed in 251 species of taxonomically unrelated plants. They were accidentally discovered in the search for an insect moulting hormone in the late 1960ies. It was believed at that time that the insect moulting hormone had been released from special prothoracic glands (PG) for the stimulation of ecdysis. Accordingly, the first ECD isolated from pupae of the silkworm, *Bombyx mori*, was named ecdysone. After identification of ecdysone, however, a number of similar ECD was found in 251 species of varous plants, occasionally in amounts surpassing the concentrations of ECD in insects more than a million-fold[1].

A long time ago, the ability to produce ECD was encountered in insect tissue and organs other than PG, such as in oenocytes, disintegrating larval muscles or pupal intestine. In addition, removal of PG from the body of a number of insect larvae had no effect on the timing of developmental events[2]. Production of ECD by numerous peripheral organs outside PG is in serious conflict with the definition of an animal hormone. Administration of ECD into vertebrate animals revealed stimulation of muscle and bone growth in Japanese quails, anabolic growth effects in mice, rat, swine, cattle and other domestic animals.These vitamin-like effects of Ec, were corroborated by pronounced pharmacological effects (anabolic, tonic, immunogenic, antiallergenic, neurogenic, metabolism stimulating) effects in human patients of the clinique[3]. Within the polar milieu of the plant system, ECD were reported to be used as a source of polar, easily mobilised sterol required for growth of plant tissue, although ECD had no property of growth hormone in plants[4]. These facts strongly imply that ECD constitute the biologically and pharmacologically very important class of the amphoteric, water and lipid soluble D-vitamins, whose pharmacological qualities have been long time neglected due to persistent belief into the PG hormone of insects[5]. The true function of insect PG depends on metabolic formation of water by combustin of dietary lipid, which is needed for larval growth[6].

REFERENCES

1. Sláma K., Romaňuk M., Šorm F.: *Insect Hormones and Bioanalogues*,Springer Wien, New York, 477pp. (1974).
2. Sláma K.: Acta Ent. Bohemoslov. *80*, 160 (1983).
3. Sláma K., Lafont R.: Eur. J. Entomol. *92*, 355 (1995).
4. Macháčková I., Vágner M., Sláma K.: Eur. J. Entomol. *92*, 309 (1995).
5. Sláma K.: Terr. Arthropod Rev. *6*, 257 (2013).
6. Sláma K., Lukáš J.: Eur. J. Entomol. *110*, 221 (2013).

ANTICANCER AND ANTIPROLIFERATIVE ACTIVITIES OF BRASSINOSTEROIDS

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The study of plant-derived compounds with effect at the molecular level has become an important approach in the selection of new agents with antitumour activity in humans. Brassinosteroids (BRs), polyhydroxylated sterol derivatives with close structural similarity to animal and insect steroid hormones are plant growth regulators representing a group of newly-discovered agents with relatively wide-ranging effects in plants. Molecular and cellular effects of natural BRs (28-homocastasterone and 24-epibrassinolide) were examined in different human cancer cell lines and in primary endothelial cells *in vitro*[1,2]. BRs caused growth inhibition, cell cycle arrest and initiation of apoptosis in many different cancer cell lines. The inhibition of proliferation and migration of human endothelial cells by BRs was demonstrated and evidences were obtained that BRs initiate cell death by apoptosis. Observed inhibition of migration and tube formation demonstrated the antiangiogenic activity of BRs[3]. These findings indicate a potential use of BRs in the prevention of metastasis development. Investigation of the mechanisms of action of BRs 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 are promising leads for the development of a new generation of potential anticancer drugs.

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REFERENCES

1. Malikova J., Swaczynova J., Kolar Z., Strnad M.: Phytochemistry *69*, 418 (2008).
2. Steigerová J., Oklešťková J., Levková M., Rárová L., Kolář Z., Strnad M.: Chem. Biol. Interact. *2010*, 487.
3. Rárová L., Zahler S., Liebl J.,Kryštof V., Sedlák D., Bartůněk P., Strnad M.: Steroids *77*, 1502 (2012).

TARGETED ANALYSIS OF NATURALLY OCCURRING BRASSINOSTEROIDS AND PLANT ECDYSTEROIDS OF BIOLOGICAL IMPORTANCE

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Brassinosteroids (BRs) are a group of extremely low abundant (fg-pg/g) naturally occurring signalling molecules with a steroidal structure (Fig. 1) belonging to plant hormones[1].

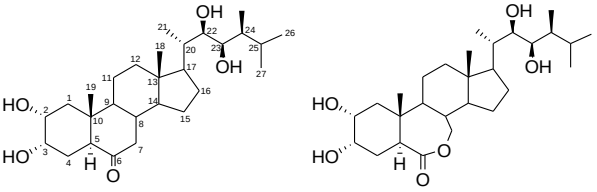

Fig. 1. **Structures of the most biologically active naturally occurring brassinosteroids**

BRs are essential growth regulators that are widespread in the plant kingdom and have structures similar to those of animal steroid hormones. Similar to their animal counterparts, BRs influence many physiological processes throughout the plant life cycle, including germination, organ elongation, timing of senescence and flowering, male fertility and increased tolerance to stress caused by temperature, water, or salinity[2]. We have developed a sensitive mass spectrometry-based method for the simultaneous profiling of eighteen brassinosteroids including biosynthetic precursors and the majority of biologically active metabolites. The extraction procedure and one-step purification based on solid-phase extraction (SPE) were optimised in combination with subsequent ultra high performance liquid chromatographic (UHPLC) analysis coupled to positive electrospray ionisation tandem mass spectrometry ((+)ESI-MS/MS) using *Brassica* flowers and *Arabidopsis* plant tissue extracts. In multiple reaction monitoring (MRM) mode, the detection limit for most of the BRs analysed ranged between 0.05 and 40 pg when using as little as 50 mg of plant tissue. This is the first analytical approach dealing with the analysis of more than eight BRs[3] without need of derivatisation.

Plant ecdysteroids (ECs) are a family of about 300 polyhydroxylated triterpenoids related in structure to the major invertebrate steroid hormone 20-hydroxyecdysone[4] (20E). ECs were originally found in animal sources and recognised as steroidal hormones controlling moulting and metamorphosis in insects[5]. Later, some of them were discovered to be present also in terrestrial plant families (ferns, gymnosperms and angiosperms).

EXPLORING NEW CHEMICAL FUNCTIONALITIES TO IMPROVE AROMATASE INHIBITION OF STEROIDS

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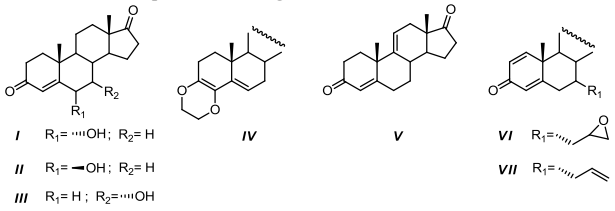
Aromatase is the only enzyme responsible for the biosynthesis of estrogens from their androgenic precursors in humans. The use of aromatase inhibitors (AIs) such as exemestane (steroidal) and letrozole (nonsteroidal) assumes

the frontline therapy for postmenopausal estrogen-dependent breast cancer. The recent structural elucidation of aromatase in complex with the natural substrate, androstenedione, gave new insights driving into the development of new AIs[1]. The importance of the C-6 and C-7 substitution[1] on steroids, for aromatase inhibition, prompted us to study the effect of the hydroxyl group in the referred positions (compounds ***I***, ***II*** and ***III***). We have also observed the need of planarity in the A-ring and A,B-ring junction[1], which can be conferred by a double bond, an epoxide function among other groups. Hence, we prepared a 3,4-dioxene derivative (compound ***IV***) and studied its anti-aromatase activity. The introduction of a further double bond in the C-ring was also explored (compound ***V***) as well as the effect of the substitution of the C-7α allyl double bond by an epoxide function (compound ***VI*** and ***VII***).

All the studied compounds demonstrated to inhibit aromatase in some extend being the percentage of inhibition, in some cases, around 90 %. From the preliminary results obtained, it can be concluded that, for aromatase inhibition, the C-6 substitution with a hydroxyl group seems to be better than the C-7 substitution, the introduction of a 3,4-dioxene group is not beneficial, the introduction of an additional double bond in the C-ring is favourable and that the substitution of the C-7α allyl double bond by an epoxide function is not quite advantageous.



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REFERENCE

1. Varela C., Amaral C., Correia da Silva G., Carvalho R. A., Teixeira N. A. A., Costa S. C., Roleira F. M. F., Tavares da Silva E. J.: Steroids *78*, 662 (2013).

MONITORING OF KEY SECONDARY METABOLITES OF ISOPRENOID NATURE IN SIBERIAN PLANTS

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Plant secondary metabolites of isoprenoid nature are of great importance[1]. We have carried out a wide-ranging study of distribution of key isoprenoids in higher plants of the South Siberia and neighboring regions within the area with geographic coordinates 48°–58° N and 77°–95° E (Russian Federation: Novosibirsk oblast, Tomsk oblast, Kemerovo oblast, Altai region, Krasnoyarsk region, Altai Republic, Republic of Khakassia; East Kazakhstan Province of the Republic of Kazakhstan).

Wild growing plants of *Apiaceae* (*Umbelliferae*), *Asteraceae* (*Compositae*), *Cupressaceae* (3 species juniper), *Lamiaceae* (*Labiatae*), and *Pinaceae* (spruce, silver fir, pine--tree, Siberian cedar, larch) families were the objects of our examination. The following groups of plant isoprenoids were studied: monoterpene hydrocarbons and oxygenated monoterpenoids as precursors for laboratory and industrial syntheses, monocyclic sesquiterpene and diterpene hydrocarbons as biosynthetic precursors of a great variety of natural sesquiterpenoids and diterpenoids, guaianolides as medically important compounds and precursors of chamazulene.

Chirospecific analysis of the plant extracts[2] showed the significant variation in enantiomeric composition of the main components with optical purity range from 99 % e.e. of (+)--enantiomer to 99 % e.e. of (–)-enantiomer depending on position of the plant population within the limits of the natural habitat. Humulene was found to be only a minor component and in most cases it was a companion of caryophyllane-type derivatives. Germacrene D was found both in (+)- and (–)- forms with variable optical purity. The express method was developed for detection of native precursor of chamazulene in plants and plant extracts. The method is suitable for detection of guaianolides both in laboratory and in field conditions. Siberian populations of *Artemisia pontica*, *A. jacutica*, and *A. macrocephala* were found to consist of several chemotypes, some chemotypes producing significant amount of guaianolides, some chemotypes not.

The research described was supported in part by Russian Foundation for Basic Research (project 13-03-00600-a).

REFERENCES

1. Tkachev A.V. *Study of plant volatiles*. Novosibirsk, 2008, 969 p. (in Russ.).
2. Tkachev A.V.: Russ. Chem. Rev. *76*, 951 (2007).

Zr-BASED APPROACH TO 5-7-6 MEMBERED RING COMPOUNDS

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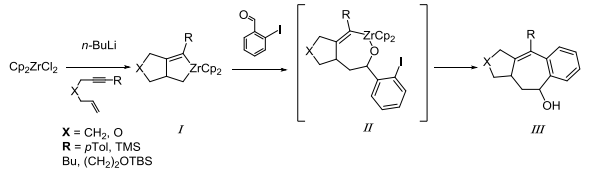
Zirconacyclopentenes, prepared by coupling a low-valent zirconocene with unsaturated hydrocarbons[1], can undergo an addition reaction to carbonyl group of various

aldehydes[2]. It is also known that substituted zirconacyclopentadienes undergo a coupling reaction with aryl halides producing fused-ring compounds[3,4]. Combining the methods mentioned above, a study was undertaken on the reaction of bicyclic zirconacyclopentenes ***I*** with commercially available halo-substituted benzaldehydes to form tricyclic compounds ***III*** (Scheme 1).



Scheme 1. **Formation of oxazirconacycle *II* and subsequent coupling reaction**

Due to its higher nucleophilicity, the C(sp^3)-Zr bond in ***I*** reacted preferentially with the carbonyl carbon of the aldehyde yielding the oxazirconacycle intermediate ***II***. The subsequent transmetallation of the remaining C(sp^2)-Zr bond with various metals salts was followed by the intramolecular cross-coupling reaction leading to the desired tricyclic product ***III*** in reasonable yields. The scope as well as mechanistic aspects of the reaction were studied in detail. This approach provides a simple and one-pot procedure for the synthesis of the fused 5-7-6 ring compounds with potential application as advanced intermediates for the syntheses of more complex molecules featuring similar carbon skeletons.

REFERENCES

1. Negishi E., Takahashi T.: Aldrichimica Acta *18*, 31 (1985).
2. Copéret C., Negishi E., Xi Z., Takahashi T.: Tetrahedron Lett. *35*, 695 (1994).
3. Takahashi T., Hara R., Nishihara Y., Kotora M.: J. Am. Chem. Soc. *118*, 5154 (1996).
4. Takahashi T., Sun W-H., Duan Z., Shen B.: Org. Lett. *2*, 1197 (2000).

TARGETING AROMATASE IN BREAST CANCER: NEW STEROIDAL OLEFINS AND EPOXIDES AS AROMATASE INHIBITORS AND STRUCTURE-ACTIVITY RELATIONSHIPS

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Breast cancer is the most common malignancy in women worldwide. Almost 70-80 % of breast cancers depend on estrogens for their development. Therefore, one efficient therapeutic approach for this disease consists in blocking estrogen production by inhibiting aromatase, the enzyme involved in its biosynthesis. The recent elucidation of the active site of aromatase revealed the importance of the establishment of a hydrogen bond with C3 oxygen atom of androstenedione, the natural substrate, and specific residues of the enzyme. Also planarity in the steroidal A-ring seems to contribute for the tight binding to the enzyme. Other studies showed that C6 substitution can be beneficial for aromatase inhibition. In this work, we designed, synthesized and evaluated the anti-aromatase activity of steroidal inhibitors obtained by introducing a C6 methyl group in steroidal hits previously reported by our group[1]. We were also interested in study the role of planarity in the A-ring and the effect of establishment of hydrogen bonding with C3 oxygen atom of the inhibitors, as important features for aromatase inhibition. For this, we have synthesized and evaluated steroidal Δ^3 and Δ^4 olefins, and its corresponding 3,4- and 4,5-epoxides, as well as a 3,4-cyclopropane analogous of the 3,4-epoxide. All the compounds revealed to be potent aromatase inhibitors, confirming that planarity in the A-ring region near of the B-ring is important for the inhibition. The 3,4-epoxide showed to be slightly more potent than the corresponding olefin, and the 3,4-cyclopropane derivative revealed to be as potent as the 3,4-epoxide. These results allowed concluding that, in the studied compounds, besides the hydrogen bond with the C3 oxygen atom, other features may contribute for the efficient inhibition of aromatase. C6 methyl steroids revealed to be also very active aromatase inhibitors, showing that this group can be well accommodated into the enzyme receptor.

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REFERENCE

1. Varela C., Tavares da Silva E., Amaral C., Correia da Silva G., Baptista T., Alcaro S., Costa G., Carvalho R. A., Teixeira N. A. A., Roleira F. M. F.: J. Med. Chem. *55*, 3992 (2012).

SYNTHETIC TRANSFORMATIONS OF *para*-MENTHANE MONOTERPENOIDS: NEW WAY TO ELABORATION OF MEDICINES FOR MENTAL HEALTH

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The compounds with the *para*-menthane framework are the most abundant natural monocyclic monoterpenoids.

These compounds possess a wide range of biological activities and affect various neuromediatory systems[1].

Based on monoterpenoids with *para*-menthane framework we synthesized a large set of new oxygen-containing compounds including heterocyclic ones[2,3].

Some of the obtained compounds demonstrated high biological activity *in vivo*. For example compound **I** possesses very promising anti-Parkinsonian activity[2]. It should be noted that Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease[4].

All eight stereoisomers and a large number of derivatives of compound **I** were synthesized in order to study structure-activity relationship. The deciding influence of the absolute configuration of the tested compounds on theirs activities was shown[2].

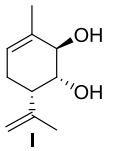

Fig. 1. **Structure of compound I**

The authors are grateful to the Russian Foundation for Basic Research for financial support (grant N 14-08-00210a).

REFERENCES

1. Pavlova A. V., Volcho K. P., Tolstikova T. G., in: *Frontiers in CNS Drug Discovery*, vol. 2, p 334. Bentham Science Publishers, Bussum 2013.
2. Ardashov O. V., Pavlova A. V., Il'ina I. V., Morozova E. A., Korchagina D. V., Karpova E. V., Volcho K. P., Tolstikova T. G., Salakhutdinov N. F.: J. Med. Chem. *54*, 3866 (2011).
3. Mikhalchenko O., Il'ina I., Pavlova A., Morozova E., Korchagina D., Tolstikova T., Pokushalov E., Volcho K., Salakhutdinov N.: Med. Chem. Res. *22*, 3026 (2013).
4. Grayson M.: Nature *466*, S1 (2010).

STEROIDS AND TRITERPENOID ACIDS IN BIOLOGICALLY ACTIVE DERIVATIVES

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Natural steroid and triterpenoid compounds are often transformed into a broad spectrum of derivatives to augment the existing biological activity and physico-chemical characteristics of the original natural products. In this search for cytotoxic and antimicrobial drugs, we have designed different conjugates of selected phytosterols, cholesterol and several triterpenoid acids. In addition, physico-chemical characteristics of selected compounds have been measured to investigate potential supramolecular behavior of the prepared compounds, and ADME parameters were calculated to support experimental data by theoretical values.

Selected natural and non-natural amino acids were employed in designing amides with selected steryl hemiesters, triterpenoid acid hemiesters formed at the C(3)-OH functionality, and amides at their C(28)-COOH functionality. Several of these compounds have represented successful structures for medicinal and supramolecular chemistry. Only a small part of these results was already published[1]. Additional analytical data will be presented at this conference.

Lipophilic derivatives of polyamines, i.e., their amides with selected triterpenoid acids or steryl hemisuccinates, the importance of which consists in enabling transportation of biologically active compounds through biomembrane, were investigated. Short-chained dicarboxylic spacers were used to bind sterols or triterpenoid acids with polyamines, enabling coincident presence of ester and amide bond in the molecules. Triterpenoid acids were also bound to polyamines through their carboxylic function by the amide bond. So far obtained data on cytotoxicity and antimicrobial activity, already published in part only[2], and will be presented at this conference.

Succinic and gluraric acid hemiesters of cholesterol and several phytosterols were used to design a series of their amides with 2-, 3- and 4-aminomethylpyridines, from which their *N*-oxides were derived. These data have been published recently[3], and the most important of them will be presented.

This work was supported by the MŠMT (projects COST LD13057, a part of the COST Network CM1106, and C26), and by the TAČR (project TA03010877).

REFERENCES

1. Šusteková J., Drašar P., Šaman D., Wimmer Z.: Molecules *16*, 9357 (2011).
2. Vida N., Svobodová H., Rárová L., Drašar P., Šaman D., Cvačka J., Wimmer Z.: Steroids *77*, 1212 (2012).
3. Bildziukevich U., Rárová L., Šaman D., Havlíček L., Drašar P., Wimmer Z.: Steroids *78*, 1347 (2013).

METATHESIS APPROACH TO NATURAL AND SYNTHETIC RETINOIDS

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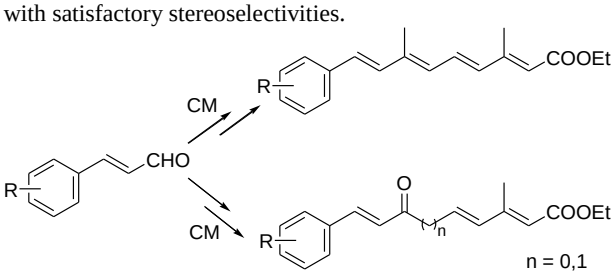
All-*trans*-retinoic acid and its natural and synthetic analogues play an essential role in a variety of biological processes such as vision, reproduction, cell differentiation, and immune response. Besides being important to normal cell function, retinoids (e.g. all-*trans*-retinoic acid,

fenretinide) show antitumor activity. Due to the application of retinoids in medicine, cosmetics, and the food industry, the search for efficient synthetic routes to these compounds is still needed. The difficulties with controlling of the olefin geometry and susceptibility of retinoids to oxidation and isomerization make their synthesis a difficult and challenging task. Nowadays, after the discovery of well-defined ruthenium and molybdenum alkylidene catalysts, olefin metathesis has provided a powerful tool for organic synthesis. It seems to be a method of choice for synthesis of vitamin A analogs as the new C–C double bonds may be formed under relatively mild conditions, which is especially important for these rather unstable compounds.

Our investigations demonstrated that cross metathesis (CM) could be a convenient and useful approach to the synthesis of retinoids[1,2]. We have developed a novel method for the preparation of aromatic retinoids from styrene derivatives which employs alternately, dienoate CM and Wittig reactions to build polyene chains. Using this strategy, several atypical retinoids were obtained in high yields and with satisfactory stereoselectivities.



Scheme 1. **Synthesis of atypical retinoids via CM strategy**

The CM approach was also successfully applied to the synthesis of ethyl all-*E*-retinoate and ethyl 9*Z*-retinoate via the C15 + C5 route.

The authors thank the Polish National Science Centre for the grant support (DEC-2011/02/A/ST5/00459).

REFERENCES

1. Maj J., Morzycki J. W., Rárová L., Wasilewski G., Wojtkielewicz A.: Tetrahedron Lett. *53*, 5430 (2012).
2. Wojtkielewicz A.: Curr. Org. Synth. *10*, 43 (2013).

ECDYSOGENS: PORPHYRIN-ECDYSTEROID COMPLEXES LIBERATING 20-HYDROXY-ECDYSONE BY INSECT METABOLISM

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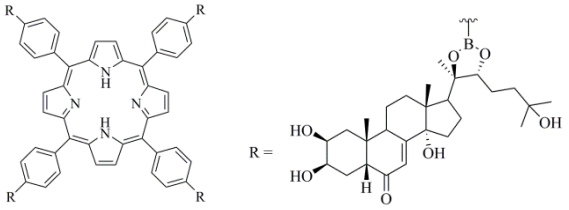
The polyhydroxylated, 6-keto derivatives of 7-dehydro-cholesterol (ecdysteroids; ECD) are essential D-vitamins[1] and important growth factors of microorganisms and plants[2]. According to a 50-year-old theory, ECD are insect hormones released from prothoracic glands, although a more recent evidence[2] shows that ECD are not hormones but homeostatic tissue factors used for reutilisation of sterol nucleus in a non-feeding stage. All ECD are effective only when injected directly into the haemolymph. We now prepared and tested for possible topical effects several porphyrin derivatives, which were coupled by hydroxyboronate method with one or more molecules of 20-hydroxyecdysone (20-E)[3]. Unfortunately, these ECD complexes were not effective when applied to insect body surface. It is more important, however, that these complexes exhibited a hitherto unknown, delayed ecdyste-roid action when injected. In larvae of the wax moth (*Galleria mellonella)*, for example, the unwanted pathophysio-logical syndromes of "Hyperecdysonism" that are currently associated with ECD injections did not occur. By contrast, injections of porphyrin-ECD complexes induced a series of perfectly timed developmental events prerequisite for normal course of the moulting process. We conclude, therefore, that these compounds generate a concentration gradient of 20-E in the body, imitating thus the naturally occurring endoge-nous ecdysteroid peaks. We are convinced that porphyrin-ECD complexes constitute a new group of metabolically activated ecdysteroid complexes (ecdysogens), liberating physiologi-cally active ecdysteroid products (20-E) from the covalently bound porphyrin complexes by endogenous metabolic pathways. These properties are analogous to the previously described[4], biochemically activated juvenoid complexes (juve-nogens), liberate biologically active products with juvenile hormone activity. Selected structural and developmental features produced by injections of porphyrin-ECD complexes in larvae and pupae of insects will be presented.



REFERENCES

1. Sláma K.: Phytoparasitica *21*, 1 (1993).
2. Sláma K.: Terr. Arthropod Rev. *6*, 1 (2013).
3. Zhylitskaya H., Zhabinskii V. N., Litvinovskaya R. P. Lettieri R., Monti D., Venanzi M., Khripach V. A., Drasar P.: Steroids *77*, 1169 (2012).
4. Sláma K., Romaňuk M.: Insect Biochem. *6*, 579 (1976).

SYNHESIS OF 26-AZABRASSINOSTEROIDS

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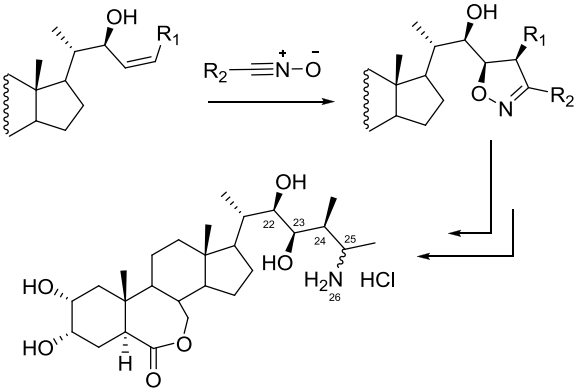
Brassinosteroids are important group of plant hormones regulating their growth, development and adaptation to stress factors. Except of natural representatives, synthetically modified analogues attract strong interest of investigators. Synthetic modifications can change physical properties (solubility, polarity etc.), stability, mode of action or even bring a new type of biological activity. A functionalization of certain positions of carbon skeleton of brassinosteroids for biotechnological applications is a common and always actual task, but the solution is often associated with problems. Here we report a synthetic way to 26-azabrassinosteroids **3**.

The key steps of the scheme are 1,3-dipolar cycloaddition of nitrile oxide to 1,2-disubstituted alkenes **1** and reductive cleavage of obtained cycloadducts **2**.



Reaction details and conditions, structure and stereochemistry of isoxazolines will be discussed in the report.

This work was supported by Grant № X13M-009 of the Belarusian Republican Foundation for Fundamental Research.

POSTERS

MODERN TECHNIQUES IN IDENTIFYING AND ANALYZING NARCOTIC DRUGS AND THEIR DERIVATIVES BASED ON LUMINESCENCE PROPERTY IN VARIOUS SAMPLES

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Need of a relatively large sample and time consuming procedures of identifying narcotic drugs in traditional analysis caused to present Fluorescence Imaging Method for fast detection of medicines and drugs. Mechanism of performance of this modern method, which is used for detecting, identifying and imaging narcotic drugs, has been based on chemical imaging that can be applied in fast analyzing of micro-medicine waste[1-2]. This technique, which requires only a few microns of sample, has been founded based on chemical imaging and results are achieved in a few seconds. Since the detecting system is very sensitive, it can be applied for fast analysis of micro-medicine waste. Hence, the system is of paramount importance for judicial purposes (coroner)[3].

Two main methods have been applied at this study for identifying and analyzing narcotic drugs and their derivatives. One method is related to obtaining chemical image of the medicines which are not visible under fluorescent test conditions. At these cases, harsh fluorescent label colors were used for testing samples. The second method is based on direct detection and auto-fluorescence chemical imaging of analyzed medicines. This method is applied for the samples which emit fluorescent from itself under test condition like lysergic acid diethylamide or LSD. Also, results of carried out studies suggest that the two methods are simple and fast and require small samples of a few micron for conducting analysis and limits of detecting are very low in pictogram efficiency.

REFERENCES

1. Fisher M., Sluszny C., Horowitz B., Bulatov V., Gridin V.V., Hassoon S., Schechter I.: Int. J. Environ. Anal. Chem. *74*, 9 (1999).
2. Villamor J. L., Bermejo A. M., Fernández P., Tabernero M. J.: Anal. Lett. *38*, 781 (2005).
3. Liu X., Nacson S., Grigoriev A., Lynds P., Pawliszyn J.: Anal. Chem. *559*, 159 (2006).

TERPENOIDS OF ESSENTIAL OILS FROM PLANTS OF ASTERACEAE, APIACEAE, LAMIACEAE FAMILIES

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The chemical compositions of essential oils of 153 species have been investigated by the GC-MS method. 86 species of essential oil compositions have been defined for the first time, identified more than 2000 components, isolated and characterized over 25 basic compounds, including, 1,8-cineole, camphor, α- and β-pinenes, limonene, sabinene, chrysanthenyl acetate, thymol, menthol, guaiol, chamazulene, etc. On the basis of above listed compounds new haloid -, sulphur-, amino- and epoxy-derivatives have been synthesized.

Asteraceae family in flora of Kazakhstan is presented by 146 genera and 883 species, including 35 endemic species. The chemical compositions of 62 species from *Artemisia*, *Achillea*, *Ajania*, *Doronicum*, *Matricaria*, *Pulicaria*, *Tanacetum*, *Stizolophus* genera have been studied by the GC-MS method.

Lamiaceae family in flora of Kazakhstan is presented by 49 genera and 247 species, including 11 endemic species. The chemical compositions of essential oils of 41 species have been investigated.

Most number of essential oil plants belongs to *Apiaceae* family. *Apiaceae* family in flora of Kazakhstan is presented by 146 genera and 883 species, including 35 endemic species. The chemical composition of essential oils of 24 species has been investigated, including composition of 13 species, that has been determined for the first time.

Data received as a result of studying essential oil composition of *Asteraceae*, *Apiaceae*, *Lamiaceae* families has shown that samples included in their components and the received derivatives have the expressed biological activity - antimicrobic, anti-inflammatory, antivirus, etc. Thus, it can be recommend to develop on their basis of phytopreparations with various spectrum of pharmacological action.

13-ARYL-SUBSTITUTED DERIVATIVES OF GROSHEIMIN

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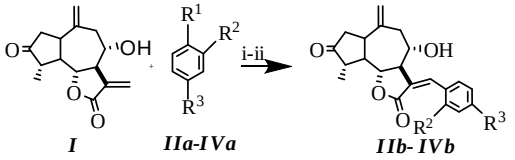
Synthesis results of natural guaianolide grosheimin ***I*** derivatives, containing the aromatic substituent in C-13 position are presented. Interaction of compound ***I*** with 2-toluene bromide ***IIa***, catalyzed by system Pd(OAc)2-(*o*-tolyl)phosphine in dimethylformamide, in the presence of cesium carbonate as the basis in working conditions[1] leads to formation of arylderivative grosheimin ***IIb***. Yield made 13% after column chromatography (in addition isolated 45 % of grosheimin ***I***). One of effective procedures of increasing yield of products of cross-combination reaction, in particular Heck reactions, is addition of tetraalkylammonium salts to reaction mixture (Jeffrey condition)[2,3]. Interaction of grosheimin ***I*** with 4-iodoanisole ***IIIa*** or 2-iodoanisole ***IVa*** at presence tetrabutylammonium bromide leads to formation of 13-aryl-substituted derivatives ***IIIb***, ***IVb*** isolated with yield of 32 and 35 % respectively.



I ***IIa-IVa*** ***IIb- IVb***

i) - $Pd(OAc)_2$, (*o*-Tol)$_3$P,Cs_2CO_3, DMF, 100-110°C, 20 h
IIb: R^1=Br; R^2=CH_3; R^3=H **(13%)**

ii) - $Pd(OAc)_2$, (*o*-Tol)$_3$P, Et_3N, TBAB, DMF, 120°C, 30 h;
IIIb: R^1=I; R^2=H; R^3=OCH_3 **(35%)**
IVb: R^1=I; R^2=OCH_3; R^3=H **(32%)**

Thus, 3 new arylderivative compounds based on grosheimin were synthesized. Structure of synthesized compounds ***IIb-IVb*** was determined on the basis of the physical and chemical constants and the spectral data (IR-, UV-, NMR ^{1}H and ^{13}C, two-dimensional spectra of NMR ^{1}H-^{1}H (COSY, NOESY) and ^{13}C-^{1}H (COSY, COLOC).

REFERENCES

1. Schultz E.E., Belovodsky A.V., Shakirov M.M., Gatilov Yu.V., Pokrovskiy A.G., Pokrovskiy M.A., Tolstikov G.A.: Chem. Natur. Comp. *2012*, 215.
2. a) Jeffery T.: Tetrahedron *52*, 10113 (1996); b) Calo V., Nacci A., Monopoli A., Detomaso A., Iliade P.: Organometallics *224*, 193 (2003).
3. a) Bonnemann H., Brinkmann R., Koppler R., Neiteler P., Richter J.: Adv. Mater. *4*, 804 (1992); b) Klingelhofer S., Heitz W., Greiner A., Oestereich S., Forster S., Antonietti M.: J. Am. Chem. Soc. *119*, 10116 (1997).

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2-(1-ISOPROPYL-4-METHYL-CYCLOHEX-3-ENYL-OXY)-ETHANOL

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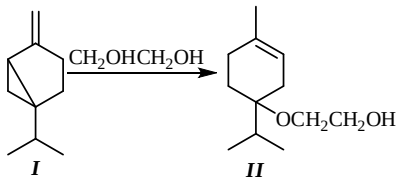
A new derivative ***II*** with composition $C_{12}H_{22}O_2$. was obtained at interaction of sabinene ***I*** with ethylene glycol at the presence of catalytic quantities of *p*-toluenesulfonic acid. The yield was 31 %.



I ***II***

In the IR spectrum of derivative ***II*** absorption band at 3436 cm^{-1} corresponding to vibrations of OH groups is present. Absorption band at 1092 cm^{-1} characterizes presence of C-O-C group. In the NMR-spectrum ^{1}H (δ, ppm, *J* (Hz)) signals are observed: 5.25 t (1H, CH), 2.05 d (2H, CH_2), 1.71 t (2H, CH_2), 1.92 t (2H, CH_2), 1.64 s (3H, CH_3), 2.10 m (1H, CH), 0.88 d (6H, $2CH_3$), 3.63 t (2H, CH_2), 3.39 t (2H, CH_2). In the NMR-spectrum ^{13}C (δ, ppm, *J* (Hz)) signals are observed: 133.97 ($C_{quarternary}$), 117.97 (CH), 26.97 (CH_2), 76.30 ($C_{quarternary}$), 27.01 (CH_2), 29.61 (CH_2), 23.11 (CH_3), 32.36 (CH), 17.24 (CH_3), 16.74 (CH_3), 62.20 (CH_2), 60.90 (CH_2).

The bioscreening determined that the sample inhibited reproduction of influenza virus A/ Tern/South Africa /1/61.

Thus, sabinene in the presence of *p*-toluenesulfonic acid with ethylene glycol forms 2-(1-isopropyl-4-methyl-cyclohex-3-enyloxy)-ethanol possessing antiviral activity.

CHLORODERIVATIVES OF BUDDLEDINE C AND THEIR SPATIAL STRUCTURE

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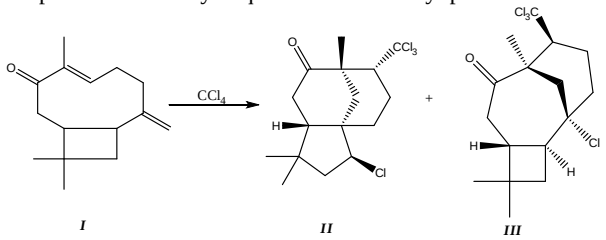
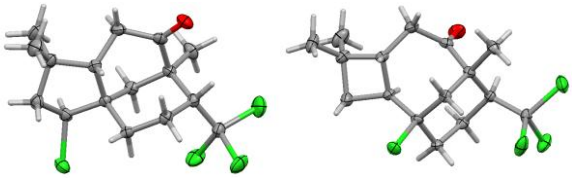
A caryophyllene type bicyclic sesquiterpene buddledine C ***I***, $C_{15}H_{22}O$, m. p. 126-128° C, $[\alpha]_D^{30}$ -314 (c=0.35, $CHCl_3$) was isolated from the air-dry raw material *Pulicaria prostrata* (Gilib.) Aschers. by the hydrodistillation method. The presence of the conjugated enone system and exomethylene bonds identifies the opportunities of aimed chemical modification of the molecule ***I***.

Two compounds clovane ***II*** and caryolane type ***III*** were obtained at interaction of buddledine C ***I*** with CCl_4 at the presence of catalytic quantities of benzoyl peroxide.



I *II* *III*



II *III*

The structure of molecules was elucidated by the X-ray method with the identification of absolute configuration of chiral centers. The molecule ***II*** has structure (2*S*)-chloro-(9*R*)-trichloromethyl-clovane-7-one and molecule ***III*** - (1*R*)-chloro- (9*S*)-trichloromethyl-caryolane-7-one.

The formation of the above mentioned reaction products with different skeletal structures is due to the conformation mobility of the caryophyllene skeleton.

EFFECT OF ESSENTIAL OIL FROM *Myrica rubra* AND SELECTED SESQUITERPENES ON ANTI-CANCER EFFICACY AND TOXICITY OF DOXORUBICIN *in vitro*

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Prolonging age of human leads to increasing number of cancer incidence. Anthracycline antibiotic doxorubicin (DOX) belongs among the most important cytostatics used in cancer therapy, but unfortunately, its cytostatic effect is frequently insufficient and severe toxicities occurs in healthy tissues[1]. Therefore, a searching for some possibilities how to increase DOX efficacy in cancer cells and minimizing associated toxicities to non-cancerous tissues is in the forefront of scientific research. *Myrica rubra*, a subtropical Asian fruit tree, is traditionaly used in the Chinese medicine[2]. Our previous experiments showed ability of *Myrica rubra* essential oil to inhibit cancer cells[3]. Our present study was designed to test possible increase of DOX efficacy via its combination with *Myrica rubra* essential oil or with individual sesquiterpenes. Experiments were realized *in vitro*. Human colon carcinoma cell line Caco2 was used as a model of cancer cells, the primary culture of rat hepatocytes serves as a model of normal cells. Cell viability was set by the NRU, MTT and BrdU tests. Relevance of interactions was determined by the CalcuSyn using the Chou-Talalay Method[4]. Obtained results showed that *Myrica rubra* oil and individual sesquiterpenes had no toxic effect on the hepatocytes. On the other hand, significant antiproliferative effect of sesquiterpenes were observed in Caco2 cell lines with IC_{50} in range 24-58μg/ml after 72h incubation. In Caco 2 cells, *Myrica rubra* oil as well as individual sesquiterpenes significantly potentiated DOX antiproliferative efficacy. Synergistic effect between DOX and sesquiterpenes was proved.

REFERENCES

1. Oktay T., Pornsak S.: J. Pharm. Pharmacol. *65*, 157 (2012).
2. He X. H., Chen L. G.: Plant Soil *262*, 229 (2004).
3. Langhasova L., Hanusova V.: Ind. Crops Prods *59*, 20 (2014).
4. Chou T.-C.: Cancer Res. *70*, 440 (2010).

EFFICIENT SYNTHESIS OF STEROIDAL RING A-FUSED QUINOLINES BY MICROWAVE IRRADIATION

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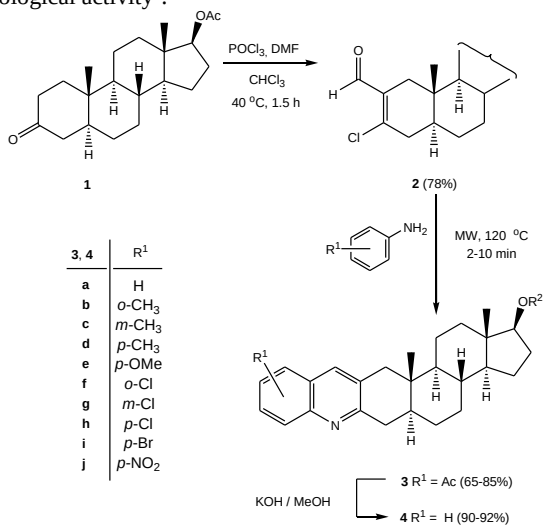
A variety of molecular hibrids or ′chimeras′ derived from steroids and other molecules have been reported so far. The incorporation of a quinoline moiety, which is also known to be the main structural building block of several pharmacologically active compounds, may have a significant influence on the chemical and stereostructural properties of the original compound resulting in useful alterations in its biological activity[1].



For the transformations, 17β-acetoxy-5α-dihydrotestosterone (**1**) was used as starting material, which was converted to β-chlorovinyl aldehyde (**2**) by Vilsmeier-Haack reaction. The MW irradiation of a mixture of **2** and aniline derivatives (1.2 equiv.) in DMF at 120 °C for 2-10 min afforded ring A-fused steroidal quinolines (**3a**–**i**) in good yields.

The products (**3a–i**) were deacetylated by KOH in MeOH to their 17β-hydroxy analogues (**4a–i**) in order to enlarge the compound library suitable for *in vitro* pharmacological studies.

The financial support by the Hungarian Scientific Research Fund (OTKA K-109107) and the New Hungary Development Plan (TÁMOP-4.2.2.A-11/1/KONV-2012-0047) is gratefully acknowledged. The work of Á. Baji was supported by a PhD Fellowship of the Talentum Fund of Richter Gedeon Plc.

REFERENCE

1. Gogoi S., Shekarrao K., Duarah A., Bora T. C., Gogoi S., Boruah R. C.: Steroids *77*, 1438 (2012).

CONJUGATES OF STEROLS AND BETULINIC ACID WITH AMINES

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Sterols and triterpenoids are essential regulating and structural substances in living organisms. They can be used for designing novel antitumor, antimicrobial and antifungal drugs. We synthesized derivatives of lanosterol, cholesterol, stigmasterol and betulinic acid for subsequent tests of the biological activity. Also several derivatives were tested for self-assembling properties.

For each steroid, a series of derivatives was obtained. In total, six series of derivatives have so far been obtained. Each series of compounds consisted of derivatives containing either a heteroaromatic motif [picolylamine derivatives and 2-(4-aminomethylphenyl)pyridine] or their *N*-oxide derivatives. The synthetic protocol was based on three generally applied synthetic stages: (a) Protection of the amino group of the aminomethyl substituted heteroaromatic compound by obtaining FMOC derivative, (b) oxidation of the heteroaromatic nitrogen by peracetic acid resulting in obtaining the corresponding *N*-oxide, and (c) removing the protection group. Summarized yields varied in the range of 37 and 66 percents. Carboxyl functionality of betulinic acid was protected by means of benzyl chloride to form the corresponding benzyl ester. From the initial steroids and protected betulinic acid, hemisuccinates, and, in case of cholesterol and stigmasterol, hemiglutarates, were prepared. The yields varied in the range of 50 and 90 percents. Modified heteroaromatic amines bearing *N*-oxide group were coupled with the sterol hemiesters by amide bond. For testing of biological activity, we obtained the target derivatives of phytosterol hemiesters with picolylamines and 2-(4-aminomethylphenyl)pyridine in the yields varying in the range of 40 and 90 percents. Betulinic acid derivative was also coupled with three Boc-protected amines: *N*-Boc diethyl amine, *N*-Boc piperazine and N^2,N^3,N^4-tris(*N*-Boc)-spermine. Subsequently, benzyl and Boc protection groups were removed.

All series of resulting derivatives were tested for cytotoxicity on the cells of human T-lymphoblastic leukemia, breast adenocarcinoma, cervical cancer, and also on normal human fibroblasts for comparison. Derivatives of betulinic acid were also tested against some microorganisms such as: *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

A financial support from the projects MSMT COST LD13057, a part of the COST Network CM1106, TACR TA03010877, and MSMT C26 is gratefully acknowledged.

THE MEDCHEMBIO CLUSTER AND ITS PROJECTS

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The MedChemBio cluster is an organization with the goal of supporting the future development of Medicinal Chemistry and Chemical Biology. The main tasks are: the creation of a platform for an information exchange, the promotion of Medicinal Chemistry and Chemical Biology in society, the development of specific projects which are on the cusp between science and industry, the development of small and medium enterprises in the fields of innovative therapeutic and diagnostic approaches, and also communication between local and European communities in these fields.

The MedChemBio cluster has become a key entity for the cooperation of academic institutions, companies, suppliers, investors, professional organizations, and production enterprises in the area of development, testing, and production of drugs, and as such it helps with the development of medicinal chemistry and chemical biology in the Czech Republic. Cluster now associates leading academic institutions (Palacky University in Olomouc, Institute of Organic Chemistry and Biochemistry in Prague and Institute of Chemical Technology Prague), major professional societies (Czech Chemical and Czech Society for Biochemistry and Molecular Biology) and a number of primarily small and medium-sized companies engaged in the fields of medicinal and biological chemistry.

The main objectives of the MedChemBio cluster can be summarized as follows: consultancy for Czech scientific workplaces in the field of technology transfer; the creation of spin-off companies; assess of intellectual property; organization of substances testing; transfer between laboratories and pilot plants; certification and legislation; investment in is the area of biologically active substances; development of the region contacts with foreign commercial partners.

This work was supported by the grant No. 5.1 SPK02/052 - Klastr MedChemBio II. The project is cofinanced from European Regional Development Fund and Ministry of Industry and Trade CR.

REFERENCE

1. http://www.medchembio.cz/ (18. 7. 2014),

STRUCTURE – CHIROPTICAL PROPERTIES RELATIONSHIP OF *CISOID* ENONES WITH α-METHYLENECYCLOPENTANONE UNIT

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In the present work, the validity of sector and helicity rules correlating the stereostructure of *cis*-enones containing 2-methylenecyclopentanone unit with the sign of nπ* Cotton effect (CE) observed in their electronic circular dichroism (ECD) spectra is assessed[1-4]. To this end, a series of model steroid *cis*-enones with five-membered ketone ring was synthesized. To investigate the scope and limitations of existing rules a combination of the ECD spectroscopy, X-ray analysis, and the time-dependent density functional theory (TD-DFT) calculations were utilized. A comparison of the experimental ECD spectra with spectra simulated by the TD-DFT calculations gave a reasonable interpretation of the nπ* CE's observed in the 360-335 nm spectral range. The results suggest that the previously articulated rules are not applicable to the investigated compounds. On the basis of comprehensive analysis of collected data, a new rule correlating perfectly the structure of studied enones with the signs of their nπ* CE was proposed. This rule correlates directly the sign of the torsion angle "b" of the cyclopentanone ring of *cis*-enone with the sign of the nπ* CE (Fig. 1).

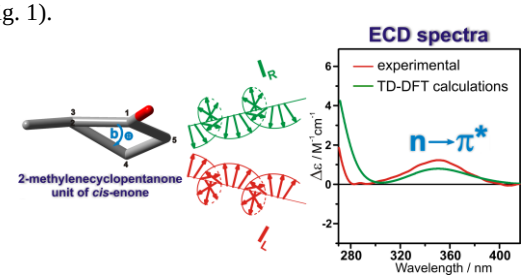

Fig. 1.

This work was supported by the National Science Centre, grant No. UMO-2011/01/B/ST5/06413. All calculations were performed at the Interdisciplinary Centre for Mathematical and Computational Modeling, University of Warsaw (ICM UW), Poland, Grants No. G 36-12 and No. G 34-15.

REFERENCES

1. Kirk D. N.: Tetrahedron *42*, 777 (1986).
2. Gawroński J. K. in: *The Chemistry of Enones* (S. Patai, Z. Rappoport Ed.), p. 55, J. Wiley & Sons Ltd 1989.
3. Frelek J., Szczepek W. J., Weiss H. P.: Tetrahedron: Assym. *4*, 411 (1993).
4. Frelek J., Szczepek W. J., Weiss H. P.: Tetrahedron: Assym. *6*, 1419 (1995).

SYNTHESIS OF BILE ACIDS-PORPHYRIN COMPOUNDS FOR SELF-ASSEMBLY STUDIES

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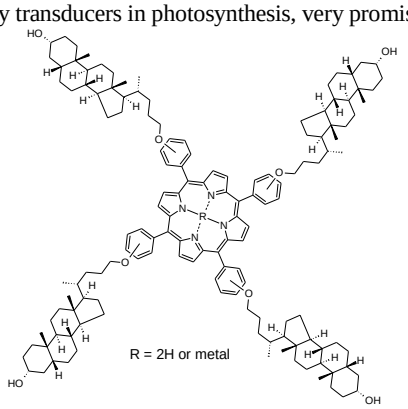
Many current electronic technologies will soon encounter both scientific and technical limits of performance and miniaturization, which is why many scientists are exploring a number of alternative device technologies, which are capable of replacing the silicon one. Novel approaches are mostly based on sp^2-hybridized carbon compounds. One of them are porphyrins, which seem to be, as natural electrical conduits, and energy transducers in photosynthesis, very promising[1].



Scheme 1. **Prepared compounds**

This work is focused on synthesis of porphyrin-steroidal conjugates for self-assembly studies. Compounds consist of two building blocks – bile acid and porphyrin. Combination of their properties (amphiphilic character of bile acid and π-π interactions of aromatic core) provides highly organized, optically active and conductive structures and all of these features can be tuned by chelation of a metal ion or by substitution on the macrocycle. We´ve investigated substitutions so far, further we want to study an influence of metal as well.

REFERENCE
1. Jurow M., Schuckman A. E., Batteas J. D., Drain C. M.: Coord. Chem. Rev. *254*, 2297 (2010).

DEUTERIUM LABELLED LIPOPHYLIC STEROID ANALOGUES. USEFUL HYDROGEN-DEUTERIUM EXCHANGE IN THE COURSE OF WITTIG REACTION

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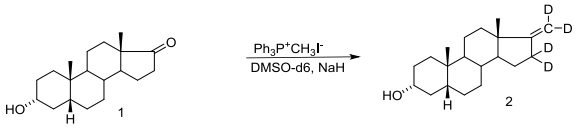
17-Methylen-5β-androstan-3α-ol derivatives labelled with four deuterium atoms were synthesized by Wittig methylenation[1]. Instead of labeled reagent, we use unlabeled triphenylphosphonium iodide in excess of d_6-dimethyl-sulfoxide. Almost quantitative hydrogen-deuterium exchange both in the position 16 and in the exomethylen group was observed in the reaction product, probably due to slow Wittig conversion of hindered carbonyl group in the position 17, competed by enolization.



Scheme 1.

Derivatives of lipophilic labeled steroid **2** were used for pharmacokinetic studies[2].

The work was supported by grant TE01020028 Center for Development of Original Drugs from the Technology Agency of the Czech Republic, grant 303/12/1465 from the Grant Agency of the Czech Republic, and RVO 61388963.

REFERENCES
1. Atkinson J. G., Fisher M. H., Horley D., Morse A. T., Stuart R. S., Synnes E.: Can. J. Chem. *43*, 1614 (1965).
2. Rambousek L., Syslová K., Valeš K., Kačer P.: FEBS J. *279*, 376 (2012).

TRITERPENE-RELATED GENES ARE DIFFERENTIALLY EXPRESSED IN RUSSETED AND WAXY VARIETIES OF APPLE

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Apple wax is a major source of pentacyclic triterpenes (ursane, oleanane and lupane series)[1,2], which are compounds of great interest for both the biomaterial and pharmaceutical industry. However, little information is available on the molecular mechanisms regulating their biosynthesis. In this study, biochemical and transcriptomic data have been collected from a panel of 20 apple cultivars presenting waxy, semi-russeted or russeted skin. Six triterpenes were identified and quantified using HPLC-DAD: 3 triterpene acids (ursolic acid (UA), oleanolic acid (OA) and betulinic acid (BA)) and 3 triterpene caffeates (betulinic acid-3-trans-caffeate (BA-transC), oleanolic acid-3-trans-caffeate (OA-transC) and betulinic acid-3-cis-caffeate (BA-cisC)). The study reveals a differential accumulation of triterpenes in apple wax according to the cultivar: (i) UA and OA were predominant in waxy apple skins as compared to russeted ones (2838.9 *vs* 1568.6

and 2044.9 *vs* 800.2 nmol/g DW) whereas BA was prominent in russeted apples as compared to waxy ones (1628.5 *vs* 686.9 nmol/g DW), (ii) the amount of triterpene-caffeates was higher in russeted apples (2901.4 *vs* 294.2 nmol/g DW). The transcription levels of 6 genes were then assessed by RT-qPCR: 4 oxydosqualene cyclases (MdOSC1, MdOSC3, MdLUP, MdbAS), a candidate squalene synthase (MdSQS) and a triterpene hydroxylase (MdTTH1). A principal component analysis was performed on triterpene contents, gene expression levels and phenotypic data and highlighted two positive correlations: (i) between expression of MdOSC1, UA contents (ursane) and waxy apples, and (ii) between expression of MdLUP, BA and BA-transC contents (lupane) and russeted apples. Our work showed for the first time the key role of MdLUP in BA accumulation. Taken together, the results from this study provide some new elements for future research on triterpene-related genes discovery and on full triterpene profile characterization in apple wax, in order to draw a more comprehensive view of triterpene pathway.

We wish to thank the Walloon Agricultural Research Center (Gembloux, Belgium) and the New Zealand Institute for Plant and Food Research (Auckland, New Zealand) for their technical support.

REFERENCES

1. André C.M., Larsen L., Burgess E., Jensen D.J., Cooney J.M., Evers D., Zhang J., Perry N.B., Laing W.A.: J. Agric. Food Chem. *61*, 2773 (2013).
2. Brendolise C., Yauk Y.K., Eberhard E.D., Wang M., Chagne D., André C.M., Greenwood D.R., Beuning L.L.: FEBS J. *278*, 2485 (2011).

MICROBIAL PRODUCTION OF 11α-HYDROXY ANDROSTENEDIONE FROM PHYTOSTEROL

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Phytosterol (***I***) is the established starting material for steroid pharmaceutical industry. Microbial transformation of phytosterols using actinobacteria is a well-known route for production of androst-4-ene-3,17-dione (***II***) and androsta-1,4-diene-3,17-dione which are the key precursors in the synthesis of different steroid drugs[1]. Effective synthesis of corticosteroids such as betamethasone, dexamethasone and other haloid-corticoids includes obtaining of androsta-4,9-diene-3,17-dione[2] which in turn may be produced from 11α--hydroxy androst-4-ene-3,17-dione (***III***). Besides, the presence of 11-hydroxy- group in steroid molecule is regarded as essential for anti-inflammatory action, and its substitution by a halogen atom does not reduce the affinity of steroid to the glucocorticoid receptor[3].

In this work, the double-step bioprocess has been developed for obtaining of ***III*** from ***I***.

The method is based on bioconversion of ***I*** by *Mycobacterium* sp. NRRL 3805B to form ***II*** followed by its regio- and stereospecific hydroxylation to ***III*** by *Aspergillus ochraceus* VKM F-830 without intermediary isolation of crystalline ***II***, - the so-called "one-pot double-stage bioprocess". Under the optimized conditions, the yield of ***III*** reached 65-68 % from 12-15 g/L of ***I***. The method has been realized in 10-L bioreactors. Downstream processing is based on the step-wise crystallization and re-crystallization from a system of polar organic solvents and provided purity of the product (***III***) of no less than 95 %. For our knowledge, microbial production of ***III*** from ***I*** was not so far reported.

The work was partly supported by the Foundation for the Assistance of Small Innovative Enterprises (FASIE), Russia, in the Framework of ERA-IB (Project: MySterI).

REFERENCES

1. Donova M. V., Egorova O. V.: Appl. Microbiol. Biotechnol. *94*, 6 (2012).
2. Huy L. D., Diep N. T., Nhung L. T. K., Pharm. Chem. J. *46*, 6 (2012).
3. Tong W. Y., Dong X.: Recent Patents on Biotechnol. *3*, 141 (2009).

PLANT GROTWTH REGULATORS OF ISOPRENOID ORIGIN

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The simple definition of a plant hormone (chemical entities are known as plant growth regulators) is that it is a molecule that at micromolar or lower concentrations acts as a messenger between plant cells. The phytohormones have the ability to regulate many aspects of plant growth and development from seed germination trough senescence and death of the plant. More than 60 years ago the first plant hormone auxin (indole derivative) was discovered. Since then five additional classes of plant growth regulators has been recognised: gibberellins, cytokinins, brassinosteroids, abscisic acid, and ethylene. Most recently strigolactones, salicylates, and jasmonates are beginning to gain acceptance as new classes as plant growth regulators. Many of these phytohormones are of isoprenoid origin. With time their commercial use for research purposes became a reality due to the OlChemIm supply advertised at the site: www.olchemim.cz. Their use in agriculture has also a great deal of potential and with time their commercial use for these

purposes may become a reality. We are opened to world-wide co-operations. We invite you to ask us for any compound of phytohormone origin, which you would be willing to use in plant hormone research. Our specialists in the Research and Development Department would be glad to synthesize new derivatives, prepare new antibodies and labelled plant growth regulators, required by our customers.

This work was supported by the grant No. TA04020547 from the Technological Agency of the Czech Republic.

REFERENCE

1. www.olchemim.cz (22. 7. 2014).

IMMUNOPHARMACOLOGICAL POTENTIAL OF STEROID SAPONINS FROM *Allium porrum*

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Saponins are noted for their versatile and significant effects on various organisms living in the ecological co-existence with their natural source-plants. Their ecological role is connected with their miscellaneous physiological activities and often depends on specific details in their chemical structure or on their quantitative content[1]. Their physiological and pharmacological activity is, however, more extensive and depends intimately on the details of their chemical structure. The wide structural variation of saponins reflects in various effects, extending from beneficial, up to toxic, depending on a wide scale of varied and complex biochemical and physiological mechanisms.

The presented contribution summarises our results achieved during screening tests of selected spirostane saponins in connection with similar testing of a series of other isoprenoids, such as ecdysteroids (2), or specific terpenoids (3).

Effects of structurally related spirostane saponins isolated from *Allium porrum* (1) on immunobiological responses triggered by lipopolysaccharide and interferon-γ were tested under in vitro conditions using murine resident peritoneal macrophages. Namely, production of nitric oxide and secretion of cytokines and chemokines were investigated. Test agents encompassed spirostanol type of saponins differing by the number and/or position of hydroxyls at the steroid part, or by number or sequence of saccharides in the glycoside part. Relation between the molecular structure and the immunobiological activity was investigated, and implication of substituents degree was assessed. The tested compounds represent solely natural substances.

Activities of leek and/or onion specific saponins from Allium species were compared with activities of other, only indirectly related ecdysteroids, which demonstrated in the same test only insignificant effect[2]. Thus, the spirostanol saponins represent a promising potency for being effective in assumed therapeutic (nutraceutic) exploitation.

REFERENCES

1. Harmatha J., Chemo-ecological role of spirostanol saponins in the interaction between plants and insects: Proc. Phytochem. Soc. Europe, *45*, 129 (2000).
2. Harmatha J., Vokáč K., Kmoníčková E., Zídek Z.: Steroids *73*, 466 (2008).
3. Harmatha J., Buděšínský M., Vokáč K., Kostecká P., Kmoníčková E., Zídek Z.: Fitoterapia *89*, 157 (2013).

HYPOLIPIDEMIC ACTIVITY OF SPINACH WASTE PRODUCTS

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Intake of vegetables is strongly related to the reduction in the exposure to risk of cardiovascular diseases and cancer. This is due to the presence of phytochemicals in these vegetables which improve the human health. One of these vegetables, having high nutritional values is *Spinacia oleraceae* L. (Spinach). The vegetable wastes could be of great commercial value. The leaves (edible part) of Spinach have been globally studied, but other organs (flowers & roots) which could be of great interest waste products are not traced enough in literature.

Aim of this work is the evaluation of the antihyper-cholesterolemic activity in order to evaluate the potentialities of the different organs (flowers & roots).

The total methanol (70 %) and the successive extractives of flowers & roots (petroleum ether, ethyl acetate and water for each organ) were preliminary tested *in vitro* for the hypolipidemic activity. The total methanol (70 %) of both organs, as the most bioactive extract, were tested *in vivo* for the antihypercholesterolemic activity. The fatty acids, sterols and triterpenes composition of flowers and roots were analyzed by GC/MS technique and the total phenolics, flavonoids and tannins contents were determined spectrophotometrically.

Results showed that the percentage of hydrocarbons in roots (69.65 %) exceeds the flowers (60.54 %) and the major hydrocarbon in flowers was hexacosane 9-octyl (7.76 %) while all hydrocarbons in roots where approximately present in equal amounts. The percentage of sterols in roots (27.87 %) was greater than flowers (20.45 %). The contents of phenolics, flavonoids and tannins in flowers are higher than roots. The activity of the total alcoholic extracts of roots is better than flowers of Spinach as antihypercholesterolemic agents. Treatment with root extract recorded significant improvement by 52.75, 209.85, 21.84, 49.26 and 29.62 % for

TC, HDL-C, LDL-C, TG and total lipids, respectively as compared to cholesterolemic rats. Treatment with flower extract recorded improvement by 47.03, 120.21, 21.03, 33.91 and 16.31% for TC, HDL-C, LDL-C, TG and total lipids, respectively when compared to the reference drug.

It could be concluded that these waste products could be of great merit commercially. The effect could be related to a synergistic effect of the chemical components: fatty acids, sterols, phenolic compounds (as flavonoids, tannins, phenolic acids).

OXIDATION OF TERPENE COMPONENTS IN *Cryptomeria japonica* BARK

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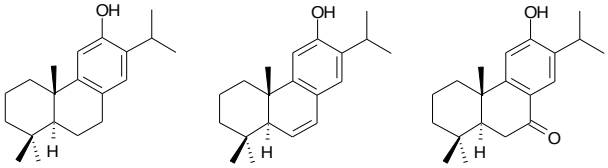
In Japanese forest, Sugi (*Cryptomeria Japonica* D. Don) has been planted in large area for building material productions. The bark of Sugi contains various terpenenoids as extractive components; however, bark is regarded as a waste material in the forestry and not utilized effectively. Sugi bark is a good source of terpenoids because some of their terpenoids components have been reported to show bioactivities, e.g. antifungal[1] and inhibition of algal growth[2].

In this study, in order to get the fundamental knowledge of the utilization of Sugi bark extract, we investigated about the oxidation products of major diterpene component in the bark.

Bark samples obtained from some varieties of Sugi were extracted by hexane and benzene (1:1=v/v) mixture after separated outer and inner barks. Extracts were analysed by GC-MS. All of Sugi bark extracts contained ferruginol as one of the main terpene components. Some components which have oxide structure of ferruginol were also detected as common components in the bark extracts.

To characterize ferruginol as a useful natural resource, we investigated its autoxidation products and process. Isolated ferruginol standard was applied to the vial and heated in oven at 85 ^{o}C for 2-32 h. After cooling the sample, ethyl acetate was added to the vials. Then, this solution was analysed by GC-MS. Ferruginol derivatives such as dehydroferruginol and sugiol were detected together with unknown component A (Fig. 1). Component A formed in early stage in the reaction, and decreased in later reaction stage. Dehydroferruginol and sugiol were stable after formation in the reaction. Dehydroferruginol and sugiol were also detected in sugi bark extracts. From the results, it was considered that component A was the intermediate of ferruginol autoxidation which led to dehydroferruginol and sugiol productions.



Ferruginol Dehydroferruginol Sugiol

Fig. 1. **Structures of ferruginol, dehydroferruginol, and sugiol**

Financial support of the work by Grant-in-Aid for JSPS Fellows (No.02604193) is greatfully acknowledged.

REFERENCES

1. Kofujita H., Fujino Y., Ota M., Takahashi K.: Holzforschung *60*, 20 (2006).
2. Saijo H., Tsuruta K., Kusumoto N., Ashitani T., Takahashi K.: J. Wood Sci. *59*, 238 (2013).

SYNTHESIS OF OXIDIZED DERIVATIVES OF EPIBRASSINOLIDE

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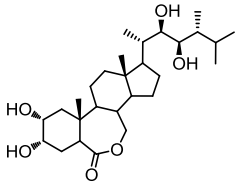
Brassinosteroids are a group of plant hormones, the study of which was started back in the late 1970s with the isolation of brassinolide and to date, about 70 related compounds have been discovered in plant sourses[1]. 24-epibrassinolide is a natural brassinosteroid which is uses in agrochemistry as a plant growth regulator. For today its application in medicine as cholesterol lowering drug is also studied. For these purposes pure samples of 24-epibrasiinolide with identified minor impurities are necessary.

In the present report, the synthesis of oxidized derivatives of 24-epibrassinolide which are its main impurities will be discussed. Their syntheses were performed by protection of appropriate hydroxyl groups followed by oxidation of unprotected alcohols or C-H bond.



24-epibrassinolide

REFERENCE

1. Bajguz A. In: *Brassinosteroids: A Class of Plant Hormones* (Hayat, S., Ahmad, A., Eds.) Springer, Dordrecht, 2011, p 1-27.

EXOGENEOUS BRASSINOSTEROIDS INDUCE CHANGES IN ECDYSTEROID CONTENT IN PLANTS

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During the examination of the involvement of exogenously applied oxysterols, 24-epibrassinolide (24E) and the 20-hydroxyecdysone (20E) in the regulation of primary photosynthetic processes we performed also the analysis of changes of the content of the above sterols in over 600 samples of tissue of greenhouse grown spinach plants. Phytoecdysteroids are present in many plant species, but their biological role is still far from being clear. Some functions of brassinosteroids as plant hormones are as well unknown. We have described that the exogenous application of 24-epibrassinolide to leaves of spinach stimulates the net photosynthetic rate[1,2]. The results are in agreement with our previous experiments with *in vitro* models, aimed at plant ecdysteroid-binding proteins identified using affinity chromatography[3,4]. The chromatography analyses with MS detection show that application of exogenous 24-epibrassinolide can influence the ecdysteroid content in plant tissues. Analysed were 20-hydroxyecdysone, polypodine B, stachysterone C, ajugasterone C and ponasterone A. 24-epibrassinolide was applied in concentration 10^{-8} M or 10^{-6} M. One group of samples was treated only in the first day, second group on 1st and 3rd day, while third group on the 1st, 3rd and 6th day after starting the experiment. The dynamics of changes in ecdysteroide content is much dependent on the age of the leaves within the same plant and on the hormone concentration.

The financial support by GACR project No. 501/11/1650 is greatly appreciated.

REFERENCES

1. Hola D., Kocova M., Rothova O., Tumova L., Kamlar M., Macek T.: Acta Physiol. Plant. *35*, 3489 (2013).
2. Rothova O., Hola D., Kocova M., Tumova L., Hnilicka F., Hnilickova H., Kamlar M., Macek T.: Steroids *85*, 44 (2014).
3. Uhlik O., Kamlar M., Kohout L., Jezek R., Harmatha J., Macek T.: Steroids *73*,1433 (2008).
4. Kamlar M., Uhlik O., Kohout L., Sanda M., Macek T.: J. Biotechnol. *150*, S490 (2010).

NOVEL FUNGAL BIOCATALYSTS FOR TRANSFORMATION OF DEOXYCHOLIC ACID

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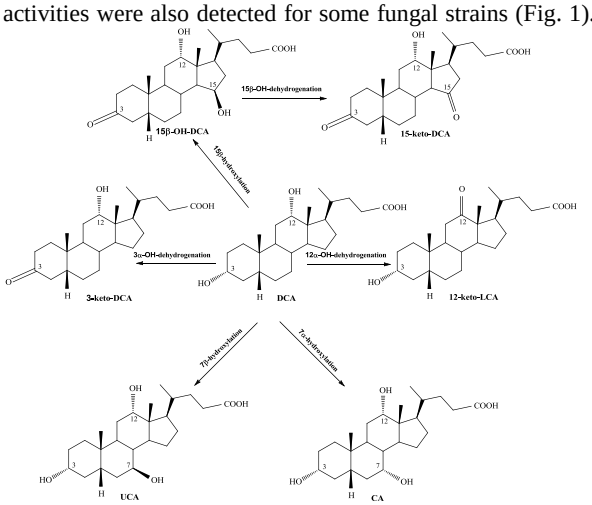
BAs are attractive raw materials for the pharmaceutical industry. It has been estimated that for each million cattle slaughtered, more than 25 tons of BA conjugates become available as by-products. Deoxycholic acid (DCA, Fig. 1) is one of the major components of these raws. Microbial transformation of DCA is of ecological relevance and essential for biotechnological production of valuable steroids.

In this work, the filamentous fungi (117 strains) from diverse phylogenetic groups have been screened toward DCA. The hydroxylating activity with formation of 7α/β-hydroxylated derivatives, - CA and ursocholic acid (UCA), correspondingly, was revealed for 34 strains of different genera (Fig. 1). A strain of *Fusarium merismoides* VKM F-2310 expressed highest level of 7β-hydroxylating activity. Under the optimized conditions the UCA yield reached 85 %.

The presence of 3α/12α-hydroxysteroid dehydrogenase, 15β-hydroxylase and 15β-hydroxysteroid dehydrogenase activities were also detected for some fungal strains (Fig. 1).



Scheme 1. **The structures of DCA and its bioconversion products formed by selected filamentous fungi strains**

The revealed broad biocatalytic potency of fungal strains toward DCA expands the knowledge of bile acids metabolism by microorganisms. The results might be suitable for preparative-scale exploitation for production of high-valued cholanic acids.

SYNTHESIS OF STEROIDAL 17-PYRAZOLYL DERIVATIVES USING VILSMEIER-HAACK REACTION

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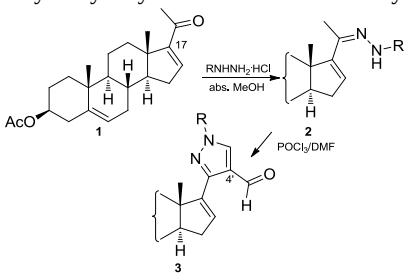
In recent decades considerable attention has been focused on the synthesis of semisynthetic steroids bearing various heteroring(s) on C-17 (ref.[1]). The modified binding ability of these derivatives to their target receptors can lead to the manifestation of some beneficial biological effects.

In continuation of our research for the synthesis of 17-*exo*-heterocyclic steroids, the aim of the present study was to synthetize novel 17-pyrazolyl derivatives in the androstane series. Condensation reaction of pregnadienolone acetate **1** with different phenylhydrazines resulted in the corresponding steroidal hydrazones **2**, which were converted to the 4-formyl pyrazoles **3** in good yields using the Vilsmeier-Haack reagent[2]. Deacetylation of **3** in basic media followed by reduction of the formyl group with $NaBH_4$ afforded 3β-hydroxy-4′-hydroxymetyl derivatives in excellent yields.



Scheme 1. **Synthesis of steroidal 17-4′-formylpyrazoles by Vilsmeier-Haack reaction**

The financial support by the Hungarian Scientific Research Fund (OTKA K-101659) and TÁMOP (TÁMOP-4.2.2.A-11/1/KONV-2012-0047) is gratefully acknowledged.

REFERENCES
1. Kovács D., Wölfling J., Szabó N., Szécsi M., Kovács I., Zupkó I., Frank É.: Eur. J. Med. Chem. *70*, 649 (2013).
2. Ivonin S. P., Kurpil B. B., Rusanov E. B., Grygorenko O. O., Volochnyuk D. M.: Tetrahedron Lett. *55*, 2187 (2014).

BIOTRANSFORMATION OF SOME STEROIDS BY *Aspergillus sydowii*

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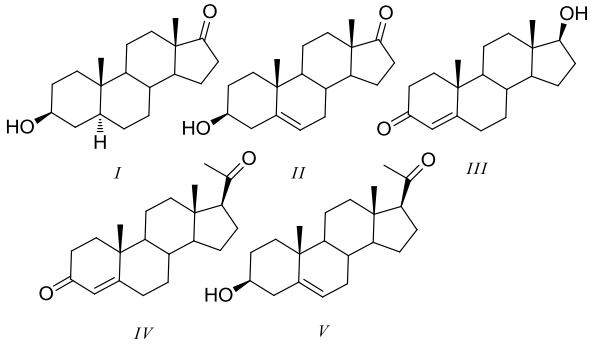
Fungal steroid biotransformations have been widely used to prepare more valuable and functionalized compounds such as steroid drugs and hormones due to their high regio- and stereoselectivity. Enormous efforts have still been made to increase the efficiency of fungal steroid biotransformations and to find new useful reactions and fungal species[1].

Aspergillus is an extremely important fungal genus concerning pathogenicity, mycotoxins, fundamental eukaryotic genetics and biotechnological exploration[2]. *Aspergillus* species are ubiquitous molds found in soil, water, and decaying materials. A few *Aspergillus* species are pathogenic to humans and animals[3].

The fungus *Aspergillus sydowii* is a mesophilic soil saprobe, a food contaminant and an opportunistic pathogen for humans[3]. As far as steroid biotransformations by *Aspergillus sydowii* are concerned, no previous work has been found in the literature.



In this work, some steroids, such as epiandrosterone ***I***, dehydroepiandrosterone ***II***, testosterone ***III***, progesterone ***IV***, and pregnenolone ***V*** were incubated with *Aspergillus sydowii* MRC 200653 for 5 days. These steroid incubations with *A. sydowii* mainly afforded hydroxylated metabolites.

The metabolites were separated by column chromatography. Structure determinations of the metabolites were performed by comparing melting points, NMR and IR spectra of starting materials with those of metabolites.

REFERENCES
1. Donova M. V., Egorova O. V.: Appl. Microbiol. Biotechnol. *94*, 1423 (2012).
2. Samson R. A., Hong S.-B., Frisvad J. C.: Med. Mycol. *44*, 133 (2006).
3. Lee S.K., Kang H.-G., Na K.-J., Han J.-I.: J. Equine Vet. Sci. *32*, 835 (2012).

THE EFFECTS OF UV-A IRRADIATION ON THE MONOTERPENE CONSTITUENTS OF CONIFEROUS NEEDLE OILS

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Coniferous needles are well known as being terpenoid-rich natural sources, and the constituents and contents of needle essential oils (EOs) vary considerable not only across

different genera but also between the species[1]. EO constituents are especially prone to degrade depend on exposure to the ambient factors such as heat, ultraviolet (UV) rays and air[2]. In terms of using EOs as pesticides, however, their high conversion properties can have an adverse impact on their activities, e.g. deterrence or repellence, because the composition of the mixtures can change over time[3]. Due to the presence of specific constituents, plenty of valid activities have been evaluated on coniferous needle oils. However, the overall effects of UV irradiation on their terpenoid constituents are still unclear. The aim of this study was to investigate the time-course effects of UV-A irradiation on the constituents of coniferous needle oils.

The needle oils of three Japanese domestic conifers, *Pinus thunbergii*, *Cryptomeria japonica* and *Thujopsis dolabrata* var. *hondae* were irradiated with UV-A by High luminance light source equipped with super-high pressure mercury lamp under ice-cold condition in open vial. After 6 hour irradiation, some *p*-menthadiene type monoterpenes (i.e. *p*-menthane olefin isomers) such as β-phellandrene and α-terpinolene in *P. thunbergii* oil; and α-terpinene, γ-terpinene and α-terpinolene in *C. japonica* and *T. dolabrata* oil completely disappeared in spite of most of the other major monoterpenes such as α-pinene, β-pinene, sabinene and limonene remained intact. Some sesquiterpene hydrocarbons such as β-caryophyllene, germacrene D and δ-cadinene also decreased rapidly. After 24 hour irradiation, monoterpene hydrocarbons and alcohols were gradually degraded, and most of other constituents remained. UV irradiation on some *p*-menthadiene standards demonstrated that most of γ-terpinene were converted into *p*-cymene, α-terpinene and α-phellandrene were converted into not only *p*-cymene but oxygenated *p*-menthanes, and α-terpinolene were converted into trace amount of *p*-cymene and numerous oxygenated monoterpenes. The time-course effects and oxygenated products of coniferous needle oil constituents by UV irradiation will be also presented.

This work was supported by the LIXIL JS Foundation (Grant no. 12-71).

REFERENCES

1. Yatagai M., Sato T., Takahashi T.: Biochem. Syst. Ecol. *13*, 377 (1985).
2. Turek C., Stintzing F.C.: Compr. Rev. Food Sci. F. *12*, 40 (2013).
3. Isman M., Miresmailli S., Machial C.: Phytochem. Rev. *10*, 197 (2011).

BIOLOGICAL ACTIVITIES OF NEW MONOHYDROXYLATED BRASSINOSTEROID ANALOGUES

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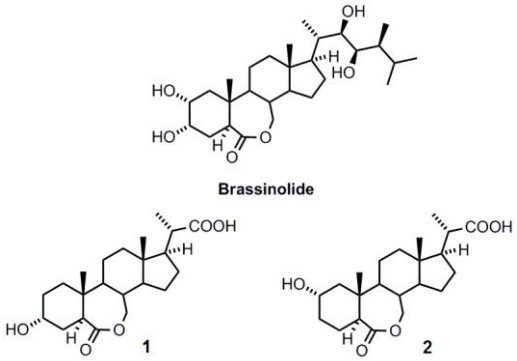
Brassinosteroids (BRs) represent a large group of plant steroids which include more than 70 structurally and functionally related compounds[1]. BRs have been found in a wide range of plant species, including higher and lower plants, and have been detected in various plant parts such as pollen, seeds, leaves, stems, roots, flowers and insect galls. They demonstrate various kinds of regulatory action on the growth and development of plants, such as the stimulation of cell enlargement and cell division, improvement of the biomass formation, yield and quality of seeds, and plant adaptability[2].



Scheme 1. **Brassinolide and monohydroxylated BRs analogues**

The aim of our study relates to the synthesis of new brassinosteroid monohydroxylated derivatives (e.g. **1** and **2**) and to study of their biological properties. The plant growth promoting activity of synthetic analogues was assayed using the bean second internode bioassay. All analogues were further analyzed with use of molecular docking of their structures into the receptor domain of kinase brassinosteroid insensitive 1 (BRI1).

This work was supported by project of the Ministry of Education,Youth and Sports CR NPUI LO1204 and by the Czech Science Foundation (GACR 14-27669P).

REFERENCES

1. Bajguz A.: Plant Physiol. Biochem. *45*, 95 (2007).
2. Clouse S. D., Sasse J. M.: Ann. Rev. Plant Physiol. Plant Mol. Biol. *49*, 427 (1998).

ISOLATION AND CHARACTERISATION OF NEW BIOACTIVE COMPOUNDS FROM *Neurolaena lobata*

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Neurolaena lobata (L.) is distributed widely in Central America and north-western parts of South America. This plant have been used in Caribbean traditional medicine for the treatment of different types of cancer, ulcers, inflammatory skin disorders, diabetes, malaria and pain of various origins. Previous studies revealed the presence of sesquiterpene lactones of the germacranolide (neurolenins A-F and lobatin A) and furanoheliangolide (lobatins B and C, 8β-isovalerianyloxy-9α-hydroxycalyculatolide and 8β-isovalerianyloxy-9α-acetoxycalyculatolide) types and some of them were reported as active compounds against inflammation and cancer *in vitro*. The aim of the present work was the isolation and identification of the new bioactive compounds from *N. lobata*.

The aerial parts of the plant were extracted with methanol. After evaporation it was subjected to solvent–solvent partition with CH_2Cl_2. The CH_2Cl_2 fraction was chromatographed by a combination of different methods, including CC, VLC, RPC and preparative TLC. The structure determinations of the isolated compounds were carried out by means of MS (ESIMS and APCIMS) and NMR (1D- and 2D-NMR) spectroscopy. The results allowed the identification of 14 sesquiterpenes, including germacranolide, eudesmanolide, eudesmane and furanoheliangolide derivatives. The isolated compounds were evaluated for their antiproliferative activities against human tumor cell lines (A2780, A431, HeLa and MCF7). Their anti-inflammatory activities were also investigated, using LPS- and TNF-α induced IL-8 expression inhibitory assay. It was observed that some of the isolated compounds showed remarkable activity.

This research was supported by the European Union and the State of Hungary, confinanced by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001"National Excellence Program" and OTKA K109846.

IMMUNOANALYTICAL SYSTEM FOR QUANTIFICATION OF SUTHERLANDIOSIDES

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Sutherlandia frutescens (Fabaceae) is a South African medicinal plant traditionally used against broad range of health problems such as internal cancers, viral diseases including AIDS, some types of inflammation, and many others. Major terpenic constituent of sutherlandia is sutherlandioside B, a cycloartane glycoside. Its presumed activity relies on inhibition of viral enzymes and anti-cancerous properties. Other structurally related cycloartane glycosides are presented in lower quantities along sutherlandioside B, mainly sutherlandiosides A, C, and D.

We developed immunoanalytical system for detection of sutherlandioside B in the format of indirect competitive ELISA. Primary polyclonal antibodies were obtained by the immunisation of rabbit with sutherlandioside B-bovine serum albumin conjugate. The secondary antibody was goat anti-rabbit IgG labelled by horseradish peroxidase. Detection limit of assay for sutherlandioside B is 0,11 ng/ml, IC50=39 ng/ml. The method analyse significant cross-reactivities with minor sutherlandiosides C and D (64 % and 147 % respectively). The results so far indicate that the method can be used to study metabolism and pharmacokinetics of sutherlandioside B.

REFERENCES

1. van Wyk B-E., Albrecht C.: J. Ethnopharmacol. *119*, 620 (2008).
2. Katerere D., Eloff J. N.: Phytother. Res. *19*, 779 (2005).
3. Johnson Q., Syce J., Nell H., Rudeen K., Folk WR.: PLoS Clin. Trials *2(4)*, e16 (2007).
4. Tai J., Cheung S., Chan E., Hasman D.: J. Ethnopharmacol. *93*, 9 (2004)
5. Fu X., Li X. C., Smilie T. J., Carvalho P., Mabusela W., Syce J., Johnson Q., Folk W., Avery M. A., Khan IA.: J. Nat. Prod. *71*, 1749 (2008).

A NOVEL ENTRY TO 10,19-NOR-VITAMIN D ANALOGS

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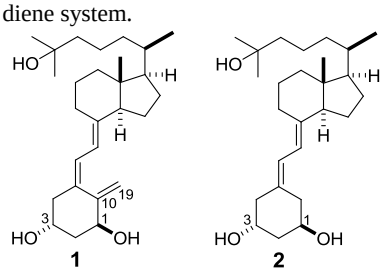
In 1990 DeLuca and coworkers have found that analogs of 1α,25-dihydroxyvitamin D_3 (**1**)[1] lacking the 19-methylene group induce differentiation and inhibit proliferation of tumor cell lines with low calcemic activity.[2] This class of compounds, known as 10,19-nor-vitamin D analogs (**2**), are of potential interest as therapeutic agents and have been synthesized by different methods including the Wittig-Horner approach,[3] the Suzuki-Miyaura couping,[4] and the Julia olefination.[5] Here we describe a new convergent approach to these compounds starting from Inhoffen-Lythgoe diol and (*R*)-carvone or (-)-quinic acid, being the major finding the convergent generation of the 10,19-nor-vitamin D diene system.



We thank the Spanish Ministry of Economy and Innovation (MEI, project SAF2010-15291) and Xunta de Galicia (project CN2012/074) for financial support. RS thanks Xunta de Galicia for an Anxeles Alvariño fellowship. RO thanks MCINN for a FPI fellowship.

REFERENCES
1. Feldman D., Pike W. S., Adams J. S. (eds.): *Vitamin D* 3rd ed. Academic Press, New York 2012.
2. Perlman K. L., Sicinski R. R., Schnoes H. K., DeLuca H. F: Tetrahedron Lett. *31*, 1823 (1990).
3. Perlman K. L., Swenson R. E., Paaren H. E., Schnoes H. K., DeLuca H. F: Tetrahedron Lett. *32*, 7663 (1991).
4. Hanazawa T., Wada T., Masuda T., Okamoto, S., Sato F.: Org. Lett. *3*, 3975 (2001).
5. Hilpert H., Wirz B.: Tetrahedron *57*, 681 (2001).

SYNTHESIS OF STEROIDAL PORPHYRIN DERIVATIVES

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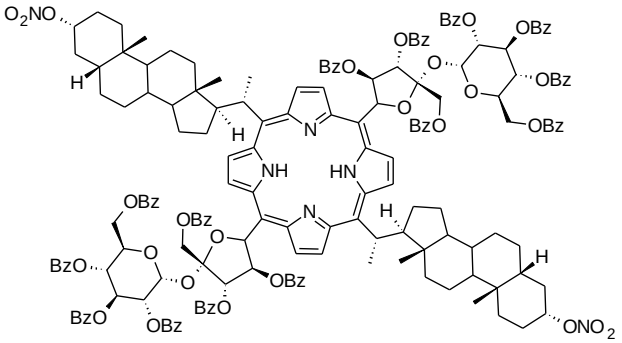
Steroid frame has been widely utilised for designing and accessing to supramolecular systems. It is rigid, chiral, with well-defined conformations, capable to enantiodiscrimination. Porphyrins, are class of aromatic compounds based on tetrapyrrole structure, occurring naturally and synthetic. A number of steroid-substituted porphyrins were obtained, most of them based on bile acid structures, estrogens, androgens and cholesterol[1-3]. Steroidal-porphyrin derivatives find application as photosensitizers for photodynamic therapy[4], in saccharide sensing[5] and anion binding[6]. Recently polyoxysterol-based porphyrin conjugates were reported[1].

We would like to present the synthesis of novel *meso*--substituted steroid-porphyrin derivative (Figure 1), based on dinor-lithocholic acid structure[7] and containing oligosaccharide units. The presence of the steroid frame may impart to the molecule possible enantiodiscrimination features, while the sucrose glycosidic groups introduce the amphiphilic character. The sucrose moieties are linked *via* "C-glycoside" bonding in order to increase resistance to enzymatic hydrolysis of the molecule.

Physicochemical properties of these derivatives, e.g. supra-assembly phenomena to form chiral suprastructures will be investigated. The potential application in photodynamic therapy will be tested as well.



Scheme 1. **Steroidal porphyrin derivative**

The authors are grateful to the Ministry of Education, Youth and Sport of the Czech Republic, for the project CZ.1.07/2.3.00/30.0060 supported by the European Social Fund.

REFERENCES
1. Zhylitskaya H.A., Zhabinskii V.N., Litvinovskaya R.P., Lettieri R., Monti D., Venanzi M, Khripach V.A.: Steroids, *77*, 1169 (2012).
2. Lorecchio C., Venanzi M., Mazzuca C., Lettieri R., Palleschi A., Nguyen Thi T. H., Cardová L., Drasar P., Monti D.: Org. Biomol. Chem., *12*, 3956 (2014).
3. Girgenti E., Ricoux R., Mahy J.-P.: Tetrahedron, *60*, 10049 (2004).
4. Sternberg E.D., Dolphin D., Tetrahedron, *54*, 4151(1998)
5. Koivukorpi J., Sievanen E., Kolehmainen E., Kral V.: Molecules, *12*, 13(2007).
6. Kolehmainen E., Koivukorpi J., Sievanen E., Kral V.: Supramol. Chem., *17*, 437(2005).
7. Drasar P., Malachowska M., Sperduto C.: Synthesis of linear ribbon derived from the lithocholic acid with shortened side chain and the method of its preparation, PTC Czech Patent Appl. PV 2014-387, 4 June 2014.

PALLADIUM-CATALYZED CROSS COUPLING OF BILE ACIDS AND 2-FURANYLBORONIC ACID. A CONVENIENT SYNTHESIS OF 24-(FURAN-2-YL)-5β-CHOLAN-24-ONES

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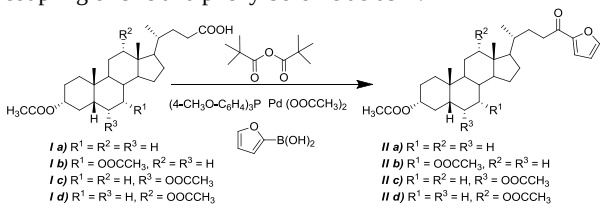
As a part of our ongoing project on the synthesis of potentially bioactive steroids, we have focused our attention in the synthesis of steroids bearing a phenyl ketone moiety in the side chain, employing the palladium catalyzed cross coupling of bile and phenylboronic acids[1-4].



Scheme 1. **Synthesis of 24-(furan-2-yl)-5β-cholan-24-ones**

We have recently directed our attention to the synthesis of steroid bearing a heteroaryl ketone moiety in the side chain. Thus, treatment of different acetylated bile acids with palladium acetate, pyvalic anhydride, tris-(4-methoxy-phenyl)-phosphine and 2-furanylboronic acid afforded the corresponding furanyl ketone in moderate yield (Scheme 1). The obtained 24-(furan-2-yl)-5β-cholan-24-ones ***(IIa-d)*** were fully characterized by their NMR spectra. NMR signals assignments were carried out with the aid of a combination of 1D and 2D NMR techniques that included ^{1}H, ^{13}C, $^{1}H–^{1}H$ COSY, Nuclear Overhauser Effect Spectroscopy (NOESY), Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC).

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REFERENCES

1. Gooßen L. J., Ghosh K.: Angew. Chem. *113*, 3566 (2001).
2. Gooßen L. J., Ghosh K.: Angew. Chem. Int. Ed. *40*, 3458 (2001).
3. Gooßen L. J., Ghosh K.: Eur. J. Org. Chem. *2002*, 3254.
4. Mayorquín-Torres M. C., Romero-Ávila M., Flores Álamo M., Iglesias-Arteaga M. A.: Steroids *78*, 1092 (2013).

SYNTHESIS OF NOVEL ANTICANCER 13α-ESTRONE CONJUGATES

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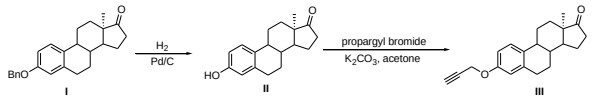
Inversion of the configuration at C-13 of natural estrone leads to epimeric compounds with the complete loss of hormonal activity. We have recently described that 13α-estrone scaffold may be a powerful strategy in the development of cytostatic or cytotoxic estrone derivatives[1].

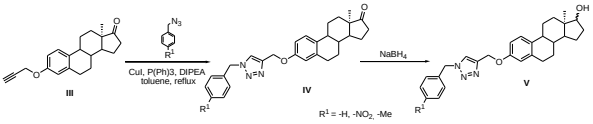
Our recent aim was to synthesize triazolyl derivatives of 13α-estrone and -estradiol and evaluation of their *in vitro* antiproliferative effect.

The alkyne function was introduced onto the phenolic hydroxyl group using propargyl bromide (Scheme 1).



Scheme 1. **Synthesis of the steroidal alkyne**

The coupling of the 13α-estrone alkyne **III** and the *p*-substituted benzyl azides was achieved via Cu(I)-catalyzed 1,3-dipolar cycloaddition reactions (CuAAC) (Scheme 2)[2].



Scheme 2. **Preparation of 13α-estrone derivatives**

The *in vitro* biological activities of the novel conjugates (**IV**, **V**) were investigated on a panel of human reproductive and skin cancer cell lines through the use of the MTT assay. Some of them exhibited a significant cytostatic effect.

The authors thank the Hungarian Scientific Research Fund (OTKA K101659), the New Hungary Development Plan (TÁMOP-4.2.2.A-11/1/KONV-2012-0047). This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'.

REFERENCES

1. Berényi A., Minorics R., Iványi Z., Ocsovszki I., Ducza E., Thole M., Messinger J., Wölfling J., Mótyán G., Mernyák E., Frank E., Schneider G., Zupkó I.: Steroids *78*, 69 (2013).
2. Liang L., Astruc D.: Coord. Chem. Rev. *255*, 2933 (2011).

LABELLED ACETOXYMETHYL ESTERS OF TRITERPENIC ACIDS

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Pentacyclic triterpenes and its derivatives have shown anti-HIV, anti-tumor and many others biological properties[1,2]. Actually, the library of semisynthetic derivatives contains more than 1000 compounds, which are fully characterized with all spectral data and their biological activities have been evaluated. For better understanding the processes in live systems and following the metabolic pathways we were synthesizing selectively labeled compounds with Hydrogen-2, Hydrogen-3, Carbon-13, Carbon-14 and Nitrogen-15.

Lupane, oleanane, ursane and taraxastane derivatives are interesting because of their different biological effects. Triterpenic acids are the well-known representatives with good cytotoxic activity. For the increasing cytotoxic activity and retain good chemical and pharmacological properties were prepared their esters. As the ester group was chosen acetoxymethyl group because the cytotoxicity of esters is comparable or even higher than the starting acid.

For this reason are Acm-esters suitable prodrugs and were chosen as candidates for the selective labeling[3]. Acetoxymethyl esters of acids with promising cytotoxicity were selectively labelled with hydrogen isotopes. The labelling was performed by reduction of ketones of previously prepared Acm-esters[4]. As the reducing agent were used $NaB[^2H]_4$ or $NaB[^3H]_4$.

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REFERENCES

1. Pisha E., Chai H., Lee I.-S., Chagwedera T. E., Farnsworth N. R., Cordell G. A., Beecher C. W. W., Fong H. H. S., Kinghorn A. D., Brown D. M., Wain M. C., Wall M. E., Hieken T. J., Das Gupta T. K., Pezzuto J. M.: Nat. Med. *1046*, 1 (1995).
2. Dzubak P., Hajduch M., Vydra D., Hustova A., Kvasnica M., Biedermann D., Markova L., Urban M., Sarek J.: Nat. Prod. Rep. *394*, 23 (2006).
3. Urban M., Šarek J., Tislerova I., Dzubak P., Hajduch M.: Bioorg. Med. Chem. *5527*, 13 (2005).
4. Vlk M., Urban M., Elbert T., Šarek J.: J. Radioanal. Nucl. Chem. *1149*, 298 (2013).

STEREOSELECTIVE SYNTHESIS OF ANDROSTENE-FUSED PYRAZOLINE DERIVATIVES BY MICROWAVE IRRADIATION

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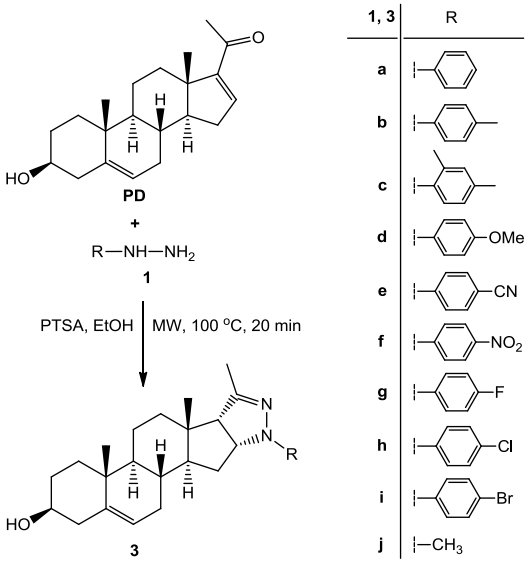
A number of different steroidal compounds containing a pyrazoline ring have been reported to exert antiproliferative activity[1]. Some of the synthetized derivatives are structurally related to natural alkaloid solanidine[2].

Novel androstenes **3** containing a pyrazoline moiety condensed to ring D of the sterane core (**3a–j**) were efficiently synthetized from **PD** with different hydrazine derivatives **1a-j**. The microwave-assisted reaction proceeded in a stereoselective manner to furnish **3a–j** in good or excellent yields (Scheme 1).



Scheme 1. **Synthesis of five-membered N,N-heterorings condensed to ring D of the sterane skeleton**

This research was supported by the EU and the State of Hungary, co-financed by the ESF in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program'. The financial support by OTKA K-109107 and TÁMOP-4.2.2.A-11/1/KONV-2012-0047 is gratefully acknowledged.

REFERENCES

1. Abdalla M. M., Al-Omar M. A., Bhat M. A., Amr A. E., Al-Mohizea A. M.: Int. J. Biol. Macromol. *50*, 1127 (2012).
2. Frank É., Mucsi Z., Zupkó I., Réthy B., Falkay G., Schneider G., Wölfling J.: J. Am. Chem. Soc. *131*, 3894 (2009).

STRUCTURAL DETERMINATION OF ISOPRENOID-TYPE ESTER GROUPS OF CHLOROPHYLL-*a* FOUND IN GREENING PROCESS

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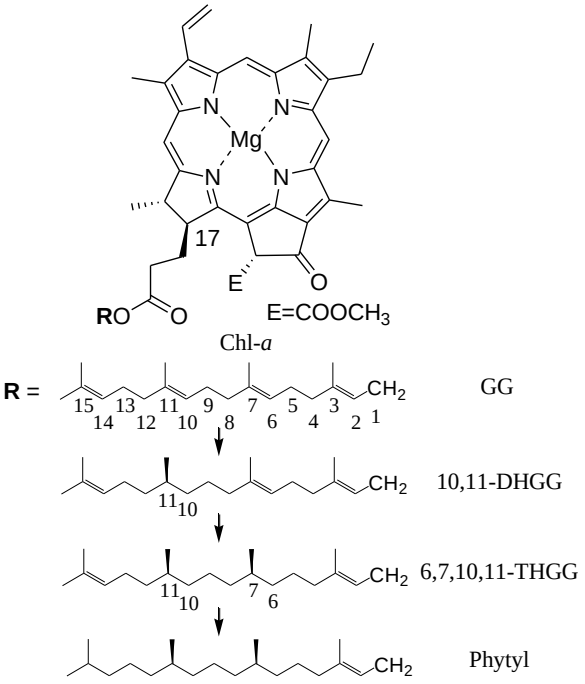
Most natural chlorophylls possess an isoprenoid ester group at the 17-propionate residue. In the final stage of chlorophyll biosynthesis, a geranylgeranyl (GG) group is regioselectively and stereoselectively reduced to a phytyl group via the intermediates, dihydrogeranylgeranyl (DHGG) and tetrahydrogeranylgeranyl (THGG) groups.



Scheme 1. **Biosynthetic reduction pathway of Chl-*a***

In our previous work, it was found that chlorophyll-*a* (Chl-*a*) extracted from a marine planktonic diatom, *Chaetoceros calcitrans* had 10,11-DHGG and 6,7,10,11-THGG groups as well as phytyl and GG groups. The biosynthetic reduction pathway of Chl-*a* was thus proposed as shown in Scheme 1.

In this study, Chl-a_{DHGG} and Chl-a_{THGG} in greening leaves of *Raphanus sativus* were identified besides Chl-a_{Phytyl} and Chl-a_{GG}. From HPLC and GLC analyses, the biosynthetic reduction pathway of isoprenoid ester moiety in Chl-*a* was confirmed.

DEVELOPMENT OF POLYCLONAL ANTIBODIES AGAINST NEUROSTEROIDS AND THEIR USE IN THE IMMUNOAFFINITY CHROMATOGRAPHY

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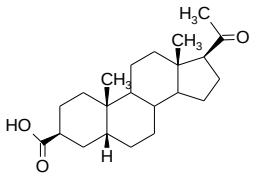
The role of neurosteroids in the pathogenesis of a number of neuropsychiatric diseases was described[1]. A number of experimental studies with animal models have shown their potential in the therapeutic treatment of several disorders of the central nervous system[2]. Therefore, it is important to have specific and sensitive method for detection of neurosteroids in various body fluids.

The aim of our study was to develop highly sensitive analytical method for identification and determination of neurosteroids, based on combination of immunoaffinity chromatography with UHPLC/MS/MS. We have prepared polyclonal antibodies against 20-oxo-5β-pregnan-3β-carboxylic acid (JB12). Antiserum against this substance was raised in rabbits immunized using JB 12 - bovine-serum albumin (BSA) conjugate. Polyclonal antibodies were cleaned up by affinity purification on protein A. The antibodies were coupled to Affi-Gel® 10 and immunoaffinity columns were prepared. The obtained antibodies were tested in enzyme-linked immunosorbent assay (ELISA) using peroxidase conjugate. The cross-reactivity study was defined on the bases of other steroids and related compounds.



Scheme 1. **Structure of 20-oxo-5β-pregnan-3β-carboxylic acid (JB12)**

This work was supported by project of the Ministry of Education, Youth and Sports CR NPUI LO1204 and by the program "Návrat" for Research, Development, and Innovations" (LK21306).

REFERENCES

1. Melcangi R.C., Garcia.Segura L.M., Mensah-Nyagan A.G.: Cell. Mol. Life. Sci. *65*, 777 (2008).
2. Pringle A.K., Schmidt W., Deans J.K., Wulfert E., Reymann K.G., Sundstrom L.E.: Eur. J. Neurosci. *18*, 117 (2003).

EXPLORING THE REACTIVITY OF *E*-23(23´)-BENZYLIDENSPIROSTANES

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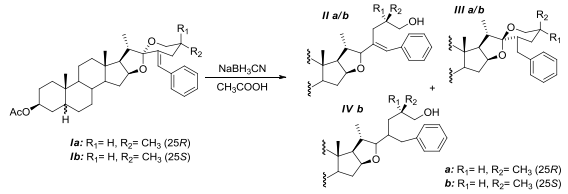
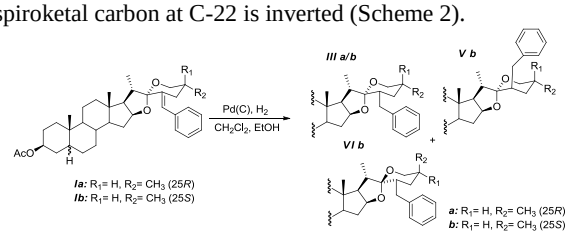
The chemistry of steroid sapogenins has concentrated the attention of organic chemist for nearly a century. Several reactions of the spiroketal side chain discovered and initially considered mere curiosities have became useful tools for the preparation of a wide variety of bioactive substances. We have recently described the synthesis of *E*-23(23´)-benzylidenspirostanes, a new family of compounds that can open a new universe of potentially useful of transformations[1]. Herein we describe our studies on the reduction of both 25*R* and 25*S* epimeric *E*-23(23´)-benzylidenspirostanes.

When the reduction of the title compound was carried out with $NaBH_3CN$ in acetic acid, the desired *E*-23(23´)-ben-zylidenfurostane ***II*** was obtanined together with the unexpected compounds ***III*** and ***IV*** (Scheme 1)[2].



Scheme 1. **Reduction of 23*E*-benzylidenspirostans with $NABH_3CN$**

On the other hand, catalytic hydrogenation of ***I*** over Pd/C afforded the expected 23-benzyl spirostanes ***III*** and ***V*** that in the case of the 25*S* series were accompanied by the unexpected compound ***VI*** in which the configuration of the spiroketal carbon at C-22 is inverted (Scheme 2).



Scheme 2. **Catalytic Reduction of 23*E*-benzylidenspirostans**

Mechanistic insights that explain the occurrence of the unexpected products, as well as the detailed characterization of all compounds are provided.

The authors acknowledge the financial support provided by Dirección General de Asuntos del Personal Académico (Project DGAPA-IN211714) and the Faculty of Chemistry (PAPIIT-5000-9063). Thanks are due to CONACYT-México for the scholarship granted to M.A.R-E.

REFERENCES

1. Ruíz-Pérez K. M., Romero-Ávila M., Tinajero-Delgado V., Flores-Álamo M., Iglesias-Arteaga M. A.: Steroids *77*, 819 (2012).
2. Ramos-Enríquez M.A., Flores-Álamo M., Iglesias-Arteaga M.A.: Tetrahedron Lett. *54*, 4401 (2013).

NEW JATROPHANE DITERPENES FROM *Euphorbia dulcis*

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Diterpenes isolated from different spurge species have unique chemical structure and promising pharmacological activity. Earlier studies on these compounds focused mainly on their skin irritant, proinflammatory and tumour promoting activities. New investigations established other pharmacological activities e.g. cytotoxic, multi-drug reversing, anti-HIV, and analgesic effects[1-3].

As part of our ongoing search for biologically active compounds from Hungarian *Euphorbia* species, we investigated the chemical constituents of *Euphorbia dulcis* L., a perennial herb native to western, southern, and central regions of Europe. Phytochemical or biological investigation on *E. dulcis* has not been reported previously.

In our study, the methanol extracts of the fresh aerial part was fractionated by liquid-liquid extraction, column chromatography, vacuum liquid chromatography, preparative TLC, and HPLC to yield four pure compounds. The structure elucidation was carried out by advanced NMR experiments (^{1}H NMR, JMOD, ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC). The isolated compounds were identified as new jatrophane diterpenes with a rare olefin linkage between C5 and C6.

This work was supported by the European Union and co-funded by the European Social Fund (TÁMOP-4.1.1.C-12/1/KONV-2012-0014).

REFERENCES

1. Vasas A., Rédei D., Csupor D., Molnár J., Hohmann J.: Eur. J. Org. Chem. *27*, 5115 (2012).
2. Witvrouw M., Pannecouque C., Fikkert V., Hantson A., Van Remoortel B., Hezareh M., De Clercq E., Brown S. J.: Antivir. Chem. Chemoth. *14*, 321 (2003).
3. Kissin I., Szallasi A.: Curr. Top. Med. Chem. *11*, 2159 (2011).

NEW APPROACHES TO CHIRAL HETEROATOMIC AUXILIARIES FROM MONOTERPENES

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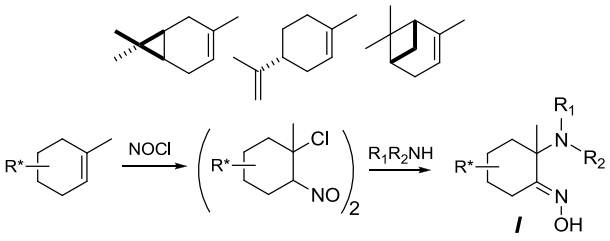
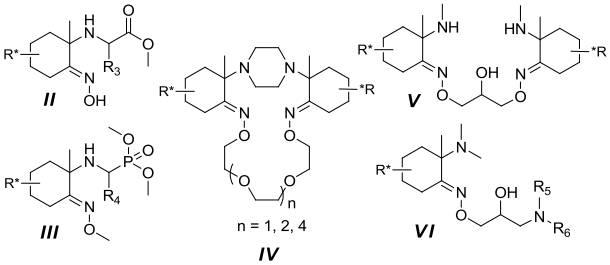
Chiral terpene auxiliaries are widely used in the modern organic synthesis[1,2]. Enantiomerically pure 3-carene, α-pinene, and limonene are among the most available primary sources of chirality, and so we employed them as starting materials in the present work. Firstly, the terpenes were transformed to α-amino oximes ***I*** according to standard procedure[3].



The oximes ***I*** were used to obtain a wide range of chiral terpene derivatives. Modification of the aminogroup led to the terpene – α-aminoacids hybrides type ***II*** as well as to α-aminophosphonic acids type ***III*** prepared by Kabachnik-Fields reaction. Another way of modification, which affected oxime group allowed us to synthesize macrocyclic compounds type ***IV*** from polyethylene glycole α,ω-dichlorides. The alcohols ***V*** and ***VI*** were produced by reaction of the initial oximes ***I*** with epichlorohydrin followed by epoxides' ring opening with nucleophiles. All the new compounds synthesized are optically active and contain a set of *N*- and *O*-functional groups that make them prospective reagents for development of novel enantioselective processes.



REFERENCES
1. Krzeminski M.P, Wojtczak M.: Tetrahedron Lett. *46*, 8299 (2005).
2. Banks M.R, Blake A.J., Brown A.R., Cadogan J.I.G., Gaur S., Gosney I., Hodgson P.K.G., Thornburn P.: Tetrahedron Lett. *35*, 489 (1994).
3. Tkachev A.V.: Ros. Khim. J. *1-2*, 42 (1998); Mendeleev Chem. J. *1998*, 42.

CYTOTOXIC TRITERPENOIDS FROM *Walsura trichostemon* Miq.

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Walsura trichostemon Miq. is naturally distributed in the evergreen forest throughout Southeast Asia, including countries such as Myanmar, Cambodia and Thailand (in North, Northeast and Southeastern). *W. trichostemon* is also used in Thai traditional medicine to treat tendon disabilities, as a staunch, for cleaning wounds, and as a treatment for hemorrhoids. In our continuing search for new anticancer agents from *W. trichostemon*, we report herein the isolation of six new cytotoxic triterpenoids, trichostemonate (**1**), 11,25-dideacetyltrichostemonate (**2**), 21,24,25-triacetyl-7-de-acetyl-6-hydroxylbrujavanone E (**3**), 7-deacetylbrujavanone E (**4**), trichostemonol (**5**) and trichostemonoate (**6**) (Fig. 1) along with seven known triterpenoids, friedelanone (**7**), β-sitosterol (**8**), melianone (**9**), 11α,20-dihydroxydammar-24-ene-3-one (**10**), sapelin E acetate (**11**), grandifolinolenenone (**12**) and *β*-sitosterol glucoside (**13**) were isolated from the roots, leaves, twigs and stem bark of this plant[1-4]. The structures of all isolated compounds were identified by interpretation of their spectroscopic data, as well as by comparison with those reported in the literature. In addition, the cytotoxicity of all isolated compounds (**1-13**) against two tumor cell lines (KB and HeLa) was also evaluated.

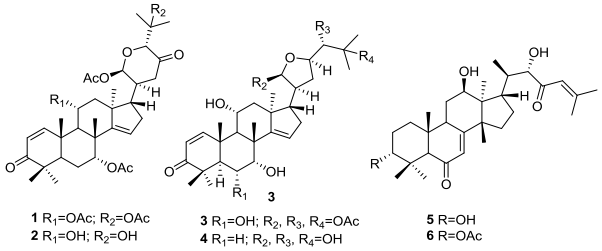

Fig. 1. **Structures of new triterpenoids (1-6) from *W. trichostemon***

Compound **1** and **10** showed significant cytotoxicity against both KB and HeLa cells (IC_{50} 3.28 and 0.93, 2.03 and 1.86 μg/mL, respectively) in the *in vitro* tumor cell panel. Compound **5** was displayed the potent cytotoxicity against only HeLa cells (IC_{50} 3.79 μg/mL), while compound **2** showed significant cytotoxicity against KB cells (IC_{50} 3.95 μg/mL). Adriamycin was used as the reference substance which exhibited activity against KB and HeLa cell

lines (IC_{50} values of 0.05 μg/mL for KB cells and 0.17 μg/mL for HeLa cells).

The authors are grateful to the Graduate School of Chulalongkorn University for Postdoctoral Fellowship (Ratchadaphiseksomphot Endowment Fund) to JS.

REFERENCES
1. Sichaem J., Aree T., Khumkratok S., Jong-Aramruang J., Tip-Pyang S.: Phytochem. Lett. 5, 665 (2012).
2. Sichaem J., Siripong P., Tip-Pyang S., Phaopongthai J.: Nat. Prod. Commun. 9, 367 (2014).
3. Sichaem J., Khumkratok S., Siripong P., Tip-Pyang S.: J. Nat. Med. 68, 436 (2014).
4. Phontree K., Sichaem J., Khumkratok S., Siripong P., Tip-pyang S.: Nat. Prod. Commun. In press, (2014).

SYNTHESIS OF A NEW α,β-UNSATURATED OXO BILE ACID

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Oxo bile acids are very interesting compounds for pharmacological studies due to the fact that they possess promotional action on the transport of some drugs through cell membranes. One possibility to further adjust hydrophilic-hydrophobic balance of oxo bile acid molecules is to introduce oxo group conjugated with double bond[1]. In this work we present synthesis of 3α-hydroxy-6-oxo-7-en--5β-cholanoic acid, a new α,β unsaturated oxo bile acid.

Bile acid derivative was synthetized starting from comercialy avaliable chenodeoxycholic acid (CDCA) in good yield. First, selective protetion of OH group in position 3 was performed then double bond was introduced by ellimination of H_2O with $POCl_3$ (ref.[2]).

The key step in this synthesis is an allylic oxidation of the olefinic bile acid with a chromium reagent in possition 6 of steroidal skeleton. Several chromium reagents known in literature were investigated, however only $CrO_3 \cdot 2Py$ in DCM (Collins reagent) gave good results[3]. In final step total deprotection with methanolic solution of sodium hydroxide followed with HCl acidification gave 3α-hydroxy-6-oxo-7-en-5β-cholanoic acid.

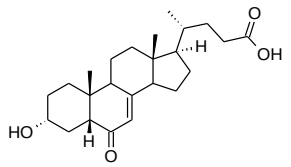

Fig. 1, **3α-Hydroxy-6-oxo-7-en-5β-cholanoic acid**

Financial support by the IPA-CBC HUSRB/1002/214/193, AP of Vojvodina Republic of Serbia 114-451-3690/2011-01 for conducting the research is gratefully acknowledged.

REFERENCES
1. Poša M.: Steroids *76*, 85 (2011).
2. Kakiyama G., Tamegai H., Lida T., Mitamura K., Ikegawa S., Goto T., Mano N., Goto J., Holz P., Hagey L., Hofmann A.: J. Lipid Res. *48*, 2682 (2007).
3. Fousteris M., Koutsourea A., Nikolaropoulos S., Riahi A., Muzart J.: J. Mol. Catal. A Chem. *250*, 70 (2006).

SYNTHESIS OF D-NOR STEROID ANALOGUES

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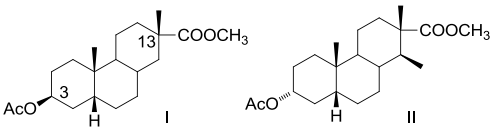
Two series of new analogues of D-nor-3α -hydroxy-5β-androstane were prepared. We started from 3β -hydroxy-5β-androsta-17-one which was converted to a steroidal indoxyl by reaction with *o*-nitrobenzaldehyde. The obtained acid was esterified with diazomethane and hydroxy group at position C-3 was acetylated.

Ozonolyzis and decarboxylation of aldehyde with Wilkinson reagent afforded steroid analogue ***I***. In the second way we started from 3α-hydroxy-5β-androsta-17-one which was converted to enolacetate. It's ozonolysis and reaction with Wilkinson reagent afforded compound ***II***.



These precursors were used for preparation of tricyclic compounds with various substituents at C-13 and hydrophilic substituent at carbon 3.

Neurophysiological activity of prepared compounds were assayed by patch clamp technique.

The work was supported by grant TE01020028 Center for Development of Original Drugs from the Technology Agency of the Czech Republic, grant 303/12/1465 from the Grant Agency of the Czech Republic, and RVO 61388963.

REFERENCES
1. Sherwin P. J., McMullan P. C., Covey D. F.: J. Med. Chem. *32*, 651 (1989).
2. Hassner A., Haddadin M. J., Catsoulacos P.: J. Org. Chem. *31*, 1363 (1966).

SYNTHESIS OF A LINEAR STEROIDAL RIBBON BASED ON DINOR-LITHOCHOLIC ACID

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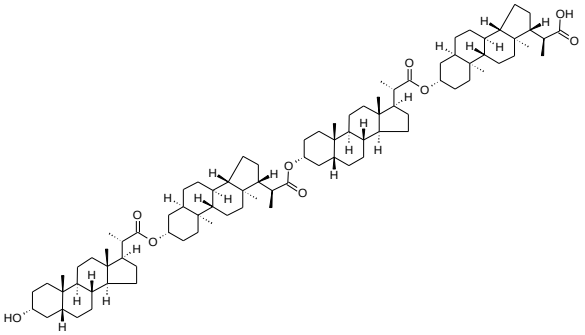
The steroidal scaffold has a great potential for the synthesis and self-assembly of larger molecular structures because of its functionalization capabilities and rigidity. These structures, consisting of several steroidal units, are very interesting due to their potential biological activity and self-assembly properties as well as for physico-chemical and biological studies. It's well known that dimeric steroids were isolated as synthetic by-products and also found in natural environment[1-2]. They showed micellar, detergent and liquid crystal behavior[3] as well as are pharmaceutically important[4]. The literature shows many examples, where steroidal acids (in most cases cholic acids) were used in various synthetic procedures[5]. Steroidal acids for their rigid and poly-functionalized framework and for their cis A-B rings connection that gives a curved profile to the molecule, forming two amphiphilic faces, are attractive material for supramolecular chemistry overall in the field of molecular and ions recognition and biomimetic catalysis[6].

We report here the synthesis of dinor-lithocholic acid tetrasteroid (Figure 1), which contains ester linkage between the steroidal units. In literature there are many examples of linear chaining of steroid units (ribbons) using esters[7] or amide[8] linkages. Amide linkage is more stable and rigid whereas the ester linked tetramer has more flexibility for induced fit interaction.



Scheme 1. **Steroidal ribbon**

The synthesis of this deprotected ester bonding-linked tetrasteroid was possible in stepwise mode using truly orthogonal protecting groups. 3-Hydroxyl group was protected by an *O*-nitro group**,** positioned orthogonally to the ester linkage. The *O*-nitro protecting group is easily removable using zinc in acidic medium[9]. A different question was the protection of the carboxylic acid moiety in position 22 of the steroid skeleton. It was proved that on this type of scaffold, the 2-(trimethylsilyl) ethyl (TMSE) ester is orthogonal to the alcohol protected by the *O*-nitro group. Further the synthesis showed that the TMSE ester was also orthogonal to the intersteroid ester bonding. TMSE group later was easily removed using TBAF in THF. For the build-up of the ester linkage, a modified condensation method using 2,6-dichlorobenzoyl chloride (DCBC) and DMAP was employed[10].

The mechanistic, physico-chemical, and sol–gel properties of the oligosteroids prepared are the subject of a forthcoming study.

The authors are grateful to the Ministry of Education, Youth and Sport of the Czech Republic, for the project CZ.1.07/2.3.00/30.0060 supported by the European Social Fund.

REFERENCES

1. Crabbe P., Zderic J.: Bull. Soc. Chim. Belg. *70*, 403 (1961).
2. Pettit G. R., Inoue M., Camano Y., Herald D. L., Arm C., Dufresne C., Christie N. D., Schmidt J. M., Doubek D. L., Krupa T. S.: J. Am. Chem. Soc, *110*, 2006 (1988).
3. McKenna J., McKenna J.M., Thornthwaite D.W.J.: J. Chem. Soc. Chem. Commun., *11*, 809 (1977)
4. Schmidt A, Beckert R, Weiß D. Tetrahedron Lett *33, 4299* (1992).
5. Davis P. A., Bonar-Law R. P., Sanders J. K. M.: Comp. Supramol. Chem. *1996*, 264.
6. Koulov V. A., Lambert T. N., Shukla R., Jain M., Boon J. M., Smith B. D., Li H., Sheppard D. N., Joos J. B,. Clare J. P., Davis P. A. Angew. Chem. Int. Ed. *42*, 4931 (2003).
7. Reschel M., Buděšínský M., Černý I., Pouzar V., Drašar P.: Collect Czech Chem Commun *67*, 1709 (2002).
8. Černý I., Budšínský M., Pouzar V, Drašar P. Collect Czech Chem Commun *66*, 933 (2001).
9. Černý I, Pouzar V, Drašar P, Havel M. Collect Czech Chem Commun *57*, 362 (1992).
10. Drašar P., Malachowska M., Sperduto C.: Synthesis of linear ribbon derived from the lithocholic acid with shortened side chain and the method of its preparation, PTC Czech Patent Appl. PV 2014-387, 4 June 2014.

AMIDES OF TRITERPENOID ACIDS

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Modern pharmaceutical industry found inspiration in natural products. Triterpenoid acids have antitumor, antiviral, cytotoxic and other biological activities. They are usually isolated from plant material like bark of birch, platane or mirabilis.

In my work I deal with preparation of conjugates of betulinic, oleanolic, platanic and ursolic acids with amino acids. Reactivity of triterpenoid acids is caused by two active groups. The C(28) carboxyl group can be directly modified with compounds containing amino group to form amide bond. At first, it is necessary to protect the C(3) hydroxyl group by a reaction with acetic anhydride in pyridine with addition of DMAP. The following step is a formation of amide bond using a short amino acid (for example glycine methyl ester) and reactive acyl chloride in the dry dichloromethane with DIPEA. The subsequent step consists in saponification of the methyl ester. The obtained product with a new carboxylic group can more easily react with hindered amino acids. The final step is a deprotection of the C(3) hydroxyl group by aqueous NaOH in THF and methanol. Transformation of the C(3) hydroxyl group requires a multistep modification. The first step consists in a formation of protection of the carboxyl group by benzyl, followed by a reaction of hydroxyl group with anhydride of dicarboxylic acid (differing in length or branching of the chain, e.g. succinic anhydride, glutaric anhydride) in pyridine with DMAP. These intermediate hemiesters were used for subsequent synthesis of conjugates with selected esters of amino acids or polyamines. Reactions were made in the dry dichloromethane, and DCC and DMAP were used as reaction promoters. The final step is a deprotection of the carboxyl group.

Biological activity and physicochemical properties of prepared compound will be presented.

A financial support from the projects MSMT COST LD13057, a part of the COST Network CM1106, TACR TA03010877, and MSMT C26) is gratefully acknowledged.

REFERENCES

1. Gerrish D., Kim I. C., Kumar D. V., Austin H., Garrus J. E., Baichwal V., Saunders M., Mckinnon R. S., Anderson M. B., Carlson R., Arranz-Plaza E. Yager M. K.: Bioorg. Med. Chem. Lett. *18*, 6377 (2008).
2. Fulda S., Friesen C., Los M., Scaffidi C., Mier W., Benedict M., Nuñez G., Krammer P. H., Peter M. E., Debatin K.-M.: Cancer Res. *57*, 4956 (1997).
3. Pezzuto J. M., Kosmeder II J. W., Xu Z.-Q., Zhou N. E., Goldsmith M. E.: US7091195B2, 15.8.2006.

PREPARATION AND ANTIPROLIFERATIVE SCREENING OF NEW TYPE OF STEROID DIMERS

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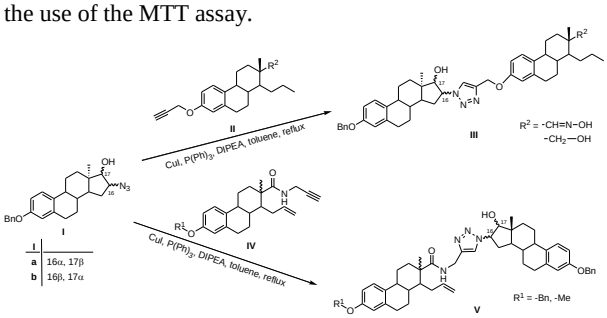
Steroids are important natural compounds, therefore different types of steroidal oligomers and conjugates were studied recently for their biological activities[1].

Our aim was to synthesize novel steroidal dimers from D-secoalkynes and 1,2-azidoalcohols of 13α- and 13β-estrone by Cu(I)-catalyzed azide/alkyne "click" reaction and to investigate their *in vitro* antiproliferative activities through the use of the MTT assay.



Scheme 1. **Preparation of steroid dimers**

The copper-catalyzed 1,3-dipolar cycloadditions (CuAAC) were carried out under classical "click" chemistry conditions (Scheme 1)[2].

The *in vitro* antiproliferative activities of the newly synthetized compounds (**III**, **V**) were determined on a panel of human adherent cancer cell lines (MCF-7, A2780, HeLa, A431). Some of the novel dimers exhibited high growth inhibitory potency against the cancer cell lines.

The authors thank the Hungarian Scientific Research Fund (OTKA K101659), the New Hungary Development Plan (TÁMOP-4.2.2.A-11/1/KONV-2012-0047). This research was supported by the EU and the State of Hungary, co-financed by the ESF in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'.

REFERENCES

1. Jurášek M., Džubák P., Sedlák D., Dvořáková H., Hajdúch M., Bartůněk P., Drašar P.: Steroids *78*, 356 (2013).
2. Liang L., Astruc D.: Coordination Chemistry Reviews *255*, 2933 (2011).

INVESTIGATION OF THE ANTIPROLIFERATIVE ACTIVITY OF SOME HIGHER MACROFUNGI

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Mushrooms have been valued for centuries not only as popular foods, but also as effective tools of traditional medicines of several countries around the world. A series of

compounds including polysaccharides, terpenes and steroids, which possess a wide range of pharmacological properties (anti-inflammatory, anticancer, and immunmodulatory activities) have been identified in many mushroom species. The aim of the present study was to investigate the potential antiproliferative activity of 17 mushrooms (16 Basidiomycetes and 1 Ascomycetes species) native to Hungary. Mushroom samples were collected in northern part of Hungary in 2013. The fresh fruiting bodies were freeze-dried, and then were extracted with methanol. The methanol extracts were subjected to solvent-solvent partition, affording hexane, chloroform and the residual extracts. After extraction with methanol, the residual mushroom materials were dried and extracted with boiling water. In the present study extracts prepared with hexane (A), chloroform (B), 50 % methanol (C) or water (D) from selected mushroom species were examined for their activity against HeLa, A431, A2780 and MCF7 cell lines. According to our results 11 extracts (out of 68) demonstrated substantial cell growth inhibitory activity (≥50 % inhibition of cell proliferation) against one or more cell lines. Among the fractions with different polarities, fraction A (hexane extracts with lipophilic constituents) and fraction B (chloroform-soluble compounds) proved to be active. The aqueous and aqueous MeOH extracts (fractions C and D, respectively) did not demonstrate notable antiproliferative effects (>50 % inhibition) against any cell line. Some mushrooms, e.g. *Fistulina hepatica*, *Gymnopus dryophilus*, *Infundibulicybe geotropa*, *Kuehneromyces mutabilis*, *Lactarius quietus* and *Lentinellus cochleatus* exerted noteworthy cytotoxic activity on one or more cell lines. These fungi are considered as promising species in the perspective of more detailed investigations. Further mycochemical studies are indispensable to identify the compounds responsible for the observed biological activity.

This work was supported by the New Hungary Development Plan Projects TÁMOP-4.1.1.C-12/1KONV-2012-0014 and TÁMOP -4.2.2.A-11/1/KONV-2012-0035.

SYNTHESIS OF NOPINANE-ANNELATED PYRIDINES

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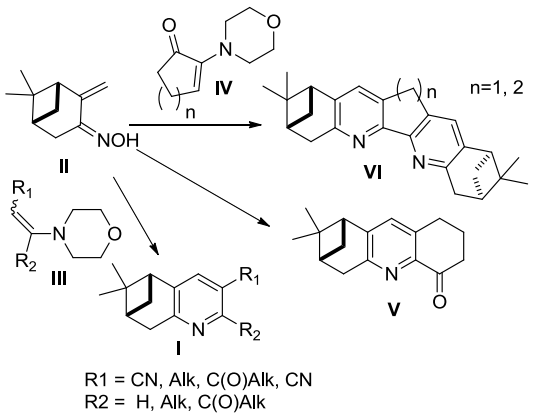
Investigation of substituted chiral pyridines gets significant attention in last two decades[1]. In our laboratory pinopyridines **I** were synthesized by reaction of pinocarvone oxime **II** with enamines **III** promoted[2] by $FeCl_3$.

Unusual results were obtained in case of enamines **IV** containing ketone functional group neighboring to the C-N enamine fragment. It was found that along with the products **V** annelation 1:1 C^2-symmetrical bipyridines **VI** were formated.



C^2-Symmetrical products **VI** prevail in case of 1) oxime **II** was taken in excess (2:1); 2) reaction was performed at high temperature (130-150 ºC); 3) reaction was promoted by $FeCl_3*6H_2O$. By this way crystalline products **VI** could be obtained with 20-25 % yields. Product **V** prepared from equimolar amounts oxime and enamine, lower temperature (100-110 ºC) and $CuCl_2*2H_2O$ as Lewis acid with 8-10 % yields.

Work was supported by projects V.46.5.4. and V.44.5.8.

REFERENCES

1. Perret-Aebi L.-E., von Zelewsky A., Dietrich-Buchecker C., Sauvage J.-P.: Angew. Chem. Int. Ed. *43*, 4482 (2004).
2. Vasilyev E.S., Agafontsev A.M., Tkachev A.V.: Synth. Comm. *44*, 1817 (2014).

CHEMICAL COMPOSITION OF ESSENTIAL OILS OF *Cymbopogon* SPECIES

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The genus *Cymbopogon* (Poaceae) contains 40 species, mostly native to the tropical regions of the world[1]. The above-ground parts of *Cymbopogon* species contain volatile oils, which are traded as 'citronella oil'. According to the European Pharmacopoeia, Citronellae aetheroleum is obtained by steam distillation from the fresh or partially dried aerial parts of *Cymbopogon winterianus* Jowitt. Main components of the official Citronellae aetheroleum are citronellal, geraniol and citronellol[2]. The extracts or essential oils of some *Cymbopogon* species are widely used in folk medicine for the treatment of digestive ailments and as flavouring material[1]. In combination with other herbal drugs they have been used for curing rheumatism, gastrointestinal and nervous disorders, and as insect repellent[3].

In our study the composition of the essential oils of *C. nervatus* (Hochst.) Ciov and *C. proximus* Stapf, collected in Sudan, were examined. The essential oils were obtained by hydrodistillation and analysed by combination of GC and GC/MS. The identification of the constituents was carried out on the basis of their retention indices and comparison of their MS data with computer library database, and with literature data[4]. The essential oil compositions of both species significantly differs from that of official Citronellae aetheroleum. The major constituent of *C. proximus* oil was piperitone (75.7 %), while main components of *C. nervatus* oil were *trans*-*p*-mentha-1(7),8-dien-2-ol (18.8 %), 1,3,5-tris(methylene)cycloheptane (17.0 %) and 1,3,8-*p*-menthatriene (14,5 %). Interestingly, this essential oil contains piperitone at only 1.5 %. These results suggest that the essential oils from *Cymbopogon* species can be readily distinguished by the analysis of the essential oil compositions, and due to the different chemical compositions of the three species they are not replaceable.

The presentation is supported by the European Union and co-funded by the European Social Fund (TÁMOP-4.1.1.C-12/1/KONV-2012-0014).

REFERENCES

1. Abushama M. F., Hassan A., Abdelgadir H., Khalid H.: Int. J. Res. Pharm. Chem. *3*, 730 (2013).
2. European Pharmacopoeia 6.0. Citronella oil 01/2008:16090.
3. Akhila A., in: *Essential oil-bearing grasses: the genus Cymbopogon*. CRC Press, Boca Raton 2010.
4. Adams R. P., in: *Identification of Essential Oil Components by GC / MS*, Allured Publishing Co. Carol Stream, Illinois USA 1995.

ISOTOPICALY LABELLED TRITERPENOIDS IN CANCER RESEARCH

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The heterogeneous family of triterpenoids, which include highly oxidized lupane, des-E-lupane, oleanane, taraxastane and others derivatives, possess a significant cytotoxic and anti-HIV activity, was named Betulinines[1-2]. Betulinines have proved multispectral cytotoxic activity on the panel of tumor lines of different histogenetical origin, including multidrug resistant[3].

Several active compounds have been synthesized, fully characterized and their mechanism of action has been described, e.g. 3β,28-diacetoxy-18-oxo-19,20,21,29,30-pentanorlupan-22-oic acid has become the most important compound of this group with IC_{50} <0.5 μmol/L. This acid causes fast and selective apoptosis of tumor cells, which is comparable to conventional anticancer drug - paclitaxel.

During the past years have been synthesized several labelled derivatives, that have been used for investigation of mechanism, labelled by deuterium, tritium, carbon-13, carbon-14 and nitrogen-15. Recent interest of our research is synthesis of heterocyclic labelled derivatives of selected acids, e.g. compounds labelled on A-ring and esters.

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REFERENCES

1. Dzubak P., Hajduch M., Vydra D., Hustova A., Kvasnica M., Biedermann D., Markova L., Urban M., Sarek J.: Nat. Prod. Rep. *394*, 23 (2006).
2. Pisha E., Chai H., Lee I.-S., Chagwedera T. E., Farnsworth N. R., Cordell G. A., Beecher C. W. W., Fong H. H. S., Kinghorn A. D., Brown D. M., Wain M. C., Wall M. E., Hieken T. J., Das Gupta T. K., Pezzuto J. M.: Nat. Med. *1046*, 1 (1995).
3. Šarek J., Klinot J., Bražinová S., Džubák P., Klinotová E., Nosková V., Křeček V., Kořínková G., Thomson J. O., Janošťáková A., Wang S., Parsons S., Fischer P. M., Zhelev N. Z., Hajdúch M.: J. Med. Chem. *5402*, 46 (2003).

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