XXI CONFERENCE ON ISOPRENOIDS

Białowieża, Poland, 23-29 September, 2005

Institute of Organic Chemistry
Polish Academy of Sciences
Warsaw

The Phytochemical Society of Europe

Institute of Chemistry
University of Białystok
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Completed 1st August, 2005

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Completed 1st August, 2005

Friday, September 23

16.00 – 21.00 Registration (Żubrówka Hotel)
18.00 – 22.00 Supper

Saturday, September 24

Morning Session:

9.00 – 9.15 Opening of the Conference

Chairperson: Marek Chmielewski

9.15 – 10.15 Amos Smith III
Evolution of a gram-scale total synthesis of the antitumor agent (+)-spongistatin 1: challenges, excitement, and frustrations

10.15 – 10.45 Adriaan J. Minnaard, Ben L. Feringa
Synthesis of linear isoprenoids using asymmetric catalysis

10.45 – 11.15 Coffee break

Chairperson: Wolfgang Steglich

11.15 – 12.15 Michel Rohmer
Biosynthesis of bacterial triterpenoids of the hopane series, a mine of novel enzyme reactions: MEP pathway for isoprenoid biosynthesis and bacteriohopanetetrol formation

12.15 – 12.45 Virginia Lanzotti
Jatrophane diterpenes as inhibitors of multidrug resistance

12.45 – 15.00 Lunch break

Afternoon Session:

Chairperson: Franca Tomé

15.00 – 15.30 Ludger A. Wessjohann
The chemistry of aromatic prenylation – from substrate synthesis to enzymatic mechanisms

15.30 – 16.00 Miroslav Strnad *et al.*
Anticancer properties of natural brassinosteroids

16.00 – 16.30 Dulcie A. Mulholland
Unusual terpenoids from African medicinal plants

16.30 – 17.00 Marek Konarzewski
Białowieża primeval forest – nature treasure of Poland

18.00 – 22.00 Welcoming reception

Sunday, September 25

Morning Session:

Chairperson: Wolfgang Kreiser

9.30 – 10.30 Philip Kocienski
New syntheses and reactions of metallated carbohydrate derivatives

10.30 – 11.00 José-Luis Giner *et al.*
Sterols of harmful marine algae: synthesis and metabolism

11.00 – 11.30 Wolfgang Steglich *et al*.
Meroterpenoids from mushrooms, a colourful group of natural products

11.30 – 11.45 Ryszard Łaźny, Aneta Nodzewska, Michał Sienkiewicz
Methodology for a solid-phase synthesis of "Daddy Longlegs" spiders defense substance

11.45 – 12.00 Meritxell Molist, Albert Ardèvol, Ladislav Kohout, Carme Brosa
Synthesis and bioactivity of androstane brassinosteroid analogs having commercially available ester side chain, selected by molecular modeling techniques

12.00 – 12.15 Eva Šťastná, Hana Chodounská
Synthesis and hydrophilic derivatization of 3α,7α-dihydroxy-5β-pregnan-20-one

12.15 – 12.30 Hans Wolf Sünnemann, Armin de Meijere
Stille-Heck cross-coupling sequences: a versatile new approach to steroids and steroid analogues

12.30 – 14.00 Lunch

14.00 – 22.00 Excursion, bonfire, supper

Monday, September 26

Morning Session:

Chairperson: Joe Connolly

9.00 – 10.00 Kenji Mori
Synthetic examination of incorrectly proposed structures of biomolecules

10.00 – 10.30 Oleg G. Kulinkovich
Recent development of the cyclopropanol methodology for the construction of methyl or methylene branched acyclic compounds

10.30 – 11.00 Ari M. P. Koskinen
Catalytic asymmetric synthesis of isoprenoids: germacradienes and limonoids

11.00 – 11.30 Coffee break

Chairperson: Hana Chodounska

11.30 – 12.00 Martin A. Iglesias-Arteaga
Steroid sapogenins still a source of new reactions and new compounds

12.00 – 12.15 Volodymyr Sashuk, Jolanta Ignatowska, Karol Grela
A fine-tuned molybdenum hexacarbonyl/phenol initiator for alkyne metathesis

12.15 – 12.30 Bartłomiej Furman
Rhodium-catalyzed intramolecular conjugate addition of vinylstannanes to 2,3-dihydro-4-pyridones. An efficient route to stereoselective construction of azabicyclic ring systems

12.30 – 15.00 Lunch break

Afternoon Session:

Chairperson: Franco Piozzi

15.00 – 16.00 Armin de Meijere *et al.*
New approaches to potentially bioactive small peptides and peptide mimetics

16.00 – 16.30 Jordan K. Zjawiony *et al.*
Salvinorin A: biologically unique diterpenoid from *Salvia divinorum*

16.30 – 16.45 Iban Jové, Cristina Peinado, Ismael Zamora, Carme Brosa
The new SHOP methodology: selecting active compounds from a database for drug discovery.

16.45 – 17.00 Jacek Młynarski, Marcin Mitura, Bartosz Rakiel
Catalytic asymmetric aldol-Tishchenko reaction catalyzed by (aminoalcohol)lanthanide complexes.

17.00 – 18.00 Poster session I (abstracts printed on odd pages)

18.00 – 19.00 Supper

20.00 – 22:00 Concert

Tuesday, September 27

Morning Session:

Chairperson: Alexander Kasal

9.00 – 10.00 Douglas F. Covey
*Ent-*steroids: chemistry and biology

10.00 – 10.30 Rita Skoda-Földes
Recent developments in palladium-catalysed carbonylation of steroids - an alternative approach to carboxylic acid derivatives

10.30 – 11.00 Nicolay V. Lukashev *et. al*
Cross-coupling reactions for steroid modification: from arylation to macrocycles syntheses

11.00 – 11.30 Coffee break

Chairperson: Carme Brosa

11.30 – 12.00 Bernd Schneider *et al.*
Biosynthesis of brassinosteroids in rye via 2,3-epoxy intermediates

12.00 – 12.30 Vladimir A. Khripach
Analysis of brassinosteroids

12.30 – 13.00 Jadwiga Frelek
Dimolybdenum method for determination of the absolute configuration of *vic*-diols **–** foundations and developments

13.00 – 15.00 Lunch break

Afternoon Session:

Chairperson: Marinus Groen

15.00 – 15.30 Biao Yu
OSW Saponins: facile synthesis toward a new type of structures with potent antitumor activities

15.30 – 16.00 Wiesław Oleszek *et al.*
Triterpene saponins in *Medicago truncatula*

16.00 – 16.15 Agnieszka Wojtkielewicz, Jacek W. Morzycki, Agnieszka Z. Wilczewska, Sławomir Wołczyński
Synthesis of analogues of a potent antitumor saponin OSW-1

16.15 – 16.30 Anna Osipova, Armin de Meijere
Synthesis and applications of new cyclopropyl-substituted isonitriles in Ugi multicomponent reactions

16.30 – 17.30 Poster session II (abstracts printed on even pages)

18.00 Banquet

Wednesday, September 28

Morning Session:

Chairperson: Janusz Jurczak

9.00 – 10.00 Masakatsu Shibasaki
Catalytic asymmetric synthesis of biologically significant natural products

10.00 – 10.30 Antonio Mouriño
Design and synthesis of a new vitamin D superagonist

10.30 – 11.00 Rafał Siciński
2-Alkylidene analogs of 19-nor-1α,25-$(OH)_2D_3$: synthesis and biological activity

11.00 – 11.30 Coffee break

Chairperson: Ladislav Kohout

11.30 – 12.00 Andrzej Kutner *et al.*
Diastereomeric and geometric analogs of vitamin D_3 - enantioselective synthesis and functional activity

12.00 – 12.15 Jan Romański, Julita Jóźwik, Christian Chapuis, Janusz Jurczak
Asymmetric 1,3-dipolar cycloadditions of chiral nitrile oxides derived from 8-phenylmenthol and (2R)-bornane-10,2-sultam to cycloalkenes

12.15 – 12.30 Libor Matyáš
Effects of 12-substituted derivatives of 5α-pregnan-20-one on $GABA_A$ receptor: synthesis and activity

12.30 – 13.00 Aede De Groot *et al.*
Steroids from carvone

13.00 Closing of the Conference

13.30 Lunch

LIST OF ONE HOUR LECTURES

EVOLUTION OF A GRAM-SCALE TOTAL SYNTHESIS OF THE ANTITUMOR AGENT (+)-SPONGISTATIN 1: CHALLENGES, EXCITEMENT, AND FRUSTRATIONS

Amos B. Smith, III

Department of Chemistry, Laboratory for Research on the Structure of Matter and the Monell Chemical Senses Center, University of Pennsylvania, Philadelphia, Pennsylvania, 19104

In 1993, the research groups of Pettit [1], Fusetani [2] and Kitagawa [3] independently disclosed the isolation of minute amounts of several congeners of the spongistatin family of bis-spiroketal macrolides that displayed extraordinary cytotoxicity (i.e., subnanomolar) against a wide variety of human cancer cell lines, including melanoma, lung, colon and brain. Named interchangeably the spongistatins, cinachyrolides, and the altohyrtins, these architecturally daunting natural products have attracted wide interest both in the synthetic and biomedical communities. The initial structural assignments of the Kitagawa group [4], including absolute stereochemical designations, were subsequently confirmed by the total syntheses of (+)-altohyrtin C [spongistatin 2 (**2**)] by Evans *et al*. [5] and (+)-altohyrtin A [spongistatin 1 (**1**)] by Kishi and coworkers [6] (Figure 1) [7]. The elegant syntheses at Harvard University demonstrated unequivocally that the altohyrtins and the spongistatins were indeed identical.

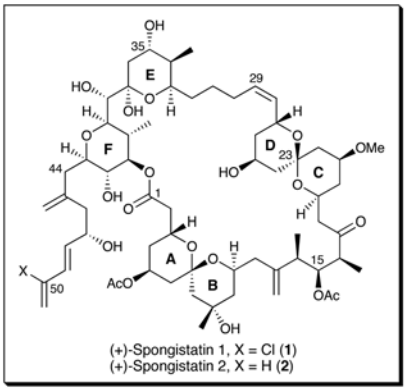


Figure 1

Intrigued both by the impressive pharmacological properties and complex architecture of the spongistatin bis-spiroketal macrolides, we initiated a synthetic program at Penn to develop a scalable route to (+)-**1**. Our enthusiasm for this program derived from our recent experience with the one-gram scale synthesis of the antitumor agent (+)-discodermolide, which was subsequently licensed by Novartis Pharmaceuticals, Inc., and with the aid of the Paterson end-game, led to clinical evaluation [8].

This lecture, as outlined below, will describe the status of our current efforts to develop a scalable (ca. one-gram) synthesis of (+)-spongistatin 1 (**1**). Towards this end, we have achieved three total syntheses of the spongistatin antitumor agents to date (Figure 2), the first in 2001 of (+)-spongistatin 2 (**2**) [9], a second in 2003 of (+)-spongistatin 1 (**1**) [10], and a third in 2004, again of (+)-spongistatin 1 (**1**) [11]. It is the latter synthesis which we believe holds the promise of providing material sufficient for preclinical evaluation. Importantly, lessons learned from our earlier ventures, as well as

those derived from the Evans and Kishi syntheses, have proven critical to the success of this program.

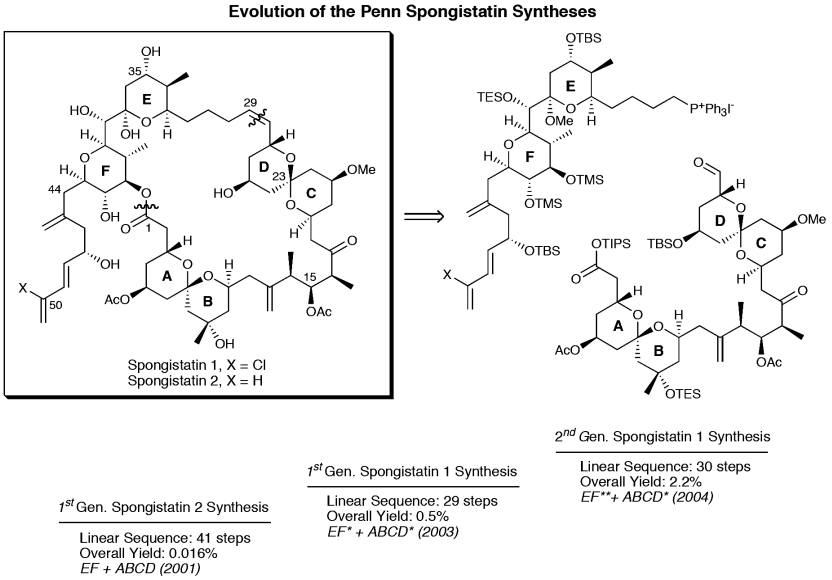
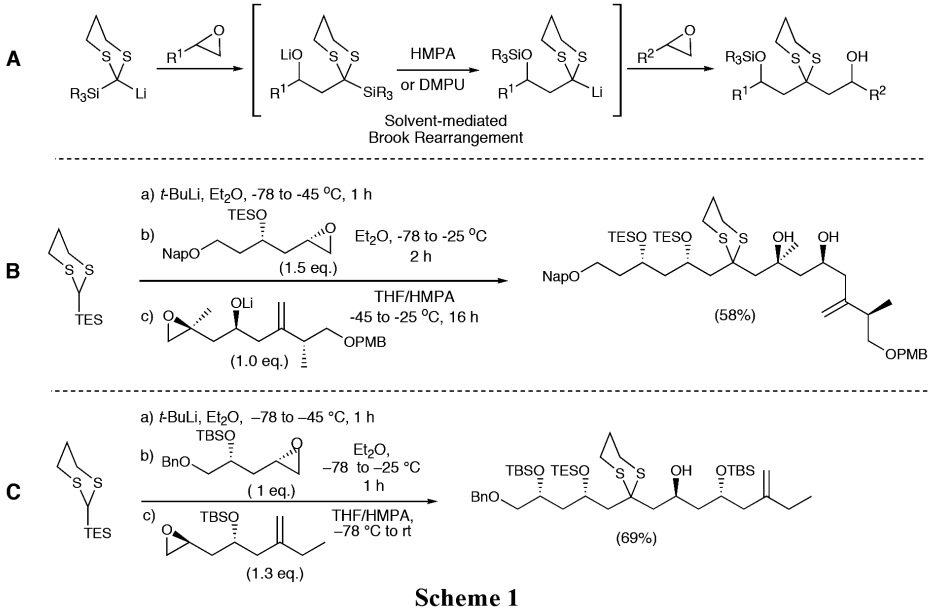


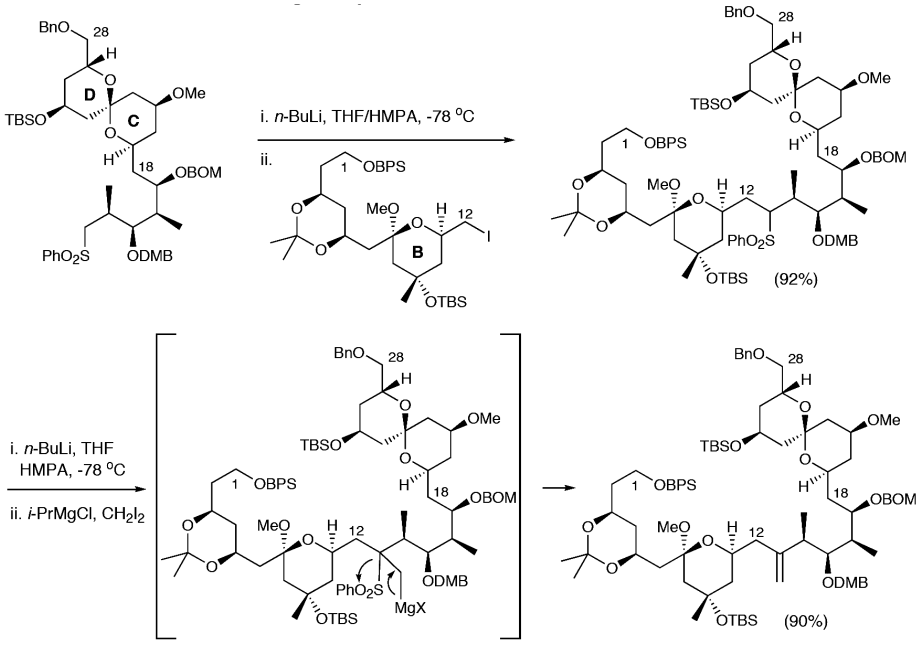
Figure 2

A central, critical feature of most successful total syntheses of architecturally complex natural products entails the efficient union of highly functionalized advanced intermediates. In this regard, we have devised and/or exploited several strategic fragment union tactics as our spongistatin program has evolved. We include here the development of the multicomponent dithiane linchpin coupling exploiting a variety of electrophiles including epoxides [12], aziridines [13], and carbonyl compounds [14], the Julia olefination, which allows fragment union with simultaneous introduction of an exo-methylene group [15], and the Petasis-Ferrier union/rearrangement tactic [16], which enables the facile construction of 2,6-*cis* disubstituted tetrahydropyran ring systems. The utility of dithiane nucleophiles as acyl anion equivalents has led to numerous applications in Smith group total syntheses [17]. More specifically, the multicomponent dithiane/epoxide linchpin coupling tactic (Scheme 1A) enables the rapid, stereoselective construction of orthogonally functionalized 1,5-diols, which are a common feature in advanced intermediates *en route* to spongistatin 1 (Scheme 1B, 1C) [18].



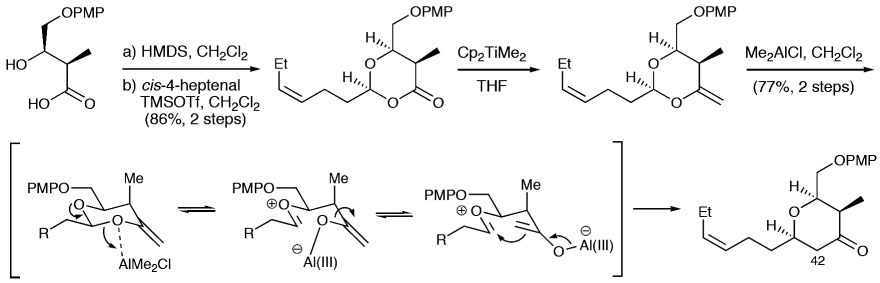
Scheme 1

Likewise, the Julia olefination/methylenation sequence [15] has enabled a strategic fragment union in the context of our efforts toward the spongistatins (Scheme 2). Of note is the overall efficiency of the two-step protocol (83%), during which a key C-C bond is formed between the CD-spiroketal sulfone and the B-ring alkyl iodide.



Scheme 2

A third transformation that is exploited with great success is the Petasis-Ferrier union/rearrangement, which enables the facile elaboration of 2,6-cis-disubstituted tetrahydropyran ring systems. Here, condensation of a β-hydroxy acid and an aldehyde, followed by methylenation of the resultant dioxanone, affords an enol acetal (Scheme 3). Treatment with Me_2AlCl then prompts a rearrangement to afford the desired tetrahydropyran intermediate.



Scheme 3

In summary, the application of each of the above mentioned strategic transformations in the context of the large-scale total synthesis of spongistatin 1 (**1**) will be described.

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[4] (a) Kobayashi, M.; Aoki, S.; Kitagawa, I. *Tetrahedron Lett.* **1994**, *35*, 1243. (b) Kobayashi, M.; Aoki, S.; Gato, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1996**, *44*, 2142.
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[7] For other total syntheses, see (a) Paterson, I.; Chen, D. Y. K.; Coster, M. J.; Acena, J. L.; Bach, J.; Gibson, K. R.; Keown, L. E.; Oballa, R. M.; Trieselman, T.; Wallace, D. J.; Hodgson, A. P.; Norcross, R. D. *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 4055. (b) Crimmins, M. T.; Katz, J. D.; Washburn, D. G.; Allwein, S. P.; McAtee, L. F. *J. Am. Chem. Soc.* **2002**, *124*, 5661. c) Heathcock, C. H.; McLaughlin, M.; Medina, J.; Hubbs, J. L.; Wallace, G. A.; Scott, R.; Claffey, M. M.; Hayes, C. J.; Ott, G. R. *J. Am. Chem. Soc.* **2003**, *125*, 12844.
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BIOSYNTHESIS OF BACTERIAL TRITERPENOIDS OF THE HOPANE SERIES, A MINE OF NOVEL ENZYME REACTIONS: MEP PATHWAY FOR ISOPRENOID BIOSYNTHESIS AND BACTERIOHOPANETETROL FORMATION

Michel Rohmer

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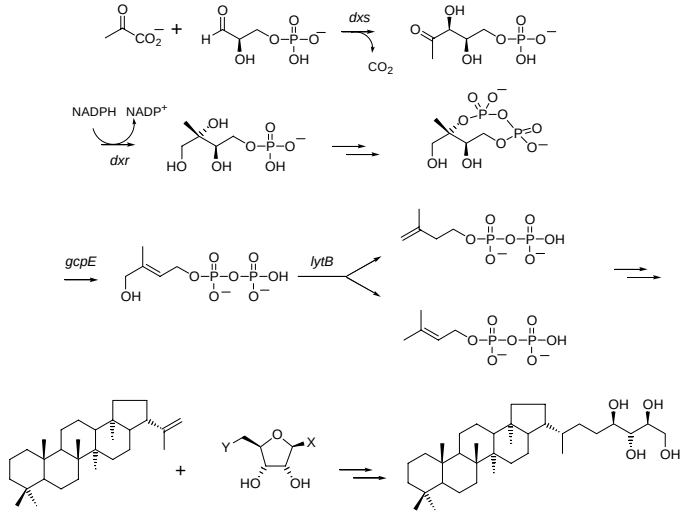
Triterpenoids of the hopane series are widespread in eubacteria, where they act as cholesterol surrogates and membrane stabilizers [1]. Their molecular fossils are ubiquitous in the organic matter of sediments [2]. Incorporation of ^{13}C labeled acetate and glucose isotopomers into bacterial hopanoids pointed out a series of novel and original enzyme reactions:

1) the methylerythritol phosphate pathway for isoprenoid biosynthesis, which is the major pathway in eubacteria, is present in the chloroplasts of all phototrophic eukaryotes, and was elucidated by a combination of labeling experiments and molecular biology methods[3],
2) the biosynthesis of the C_{35} bacteriohopane skeleton characterized by a carbon/carbon bond between a triterpene moiety and C-5 of a D-ribose derivative [4],
3) methylation of non-activated carbon atoms (C-2 and C-3) of the hopane ring A [5],
4) formation from a D-glucose derivative of a novel type of carbapseudopentose [6].



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NEW SYNTHESES AND REACTIONS OF METALLATED CARBOHYDRATE DERIVATIVES

Philip Kocienski

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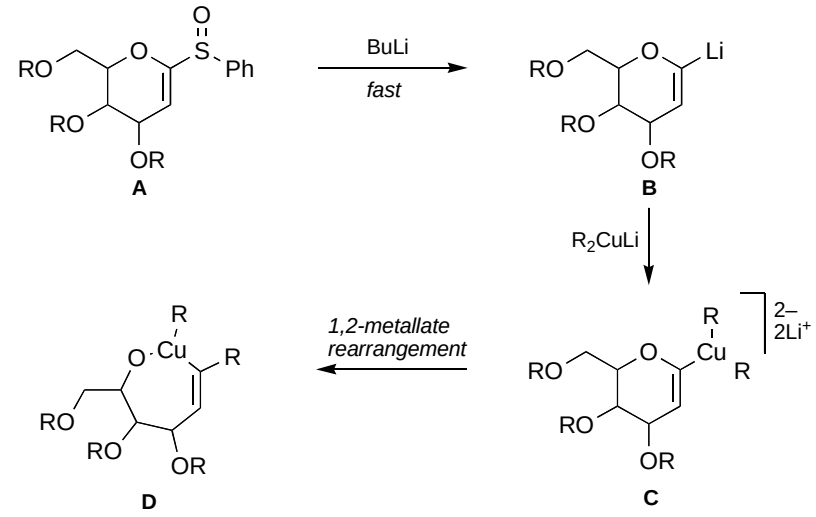
Sulfoxide-lithium exchange provides a convenient and general method for the synthesis of metallated glycals **B**. The 1,2-metallate rearrangement of higher order cuprates **C** provides a powerful method for the appendage of carbon chains to carbohydrate derivatives. The chemistry is exemplified by a synthesis of the fungal cerebroside derivative **E.**



SYNTHETIC EXAMINATION OF INCORRECTLY PROPOSED STRUCTURES OF BIOMOLECULES

Kenji Mori

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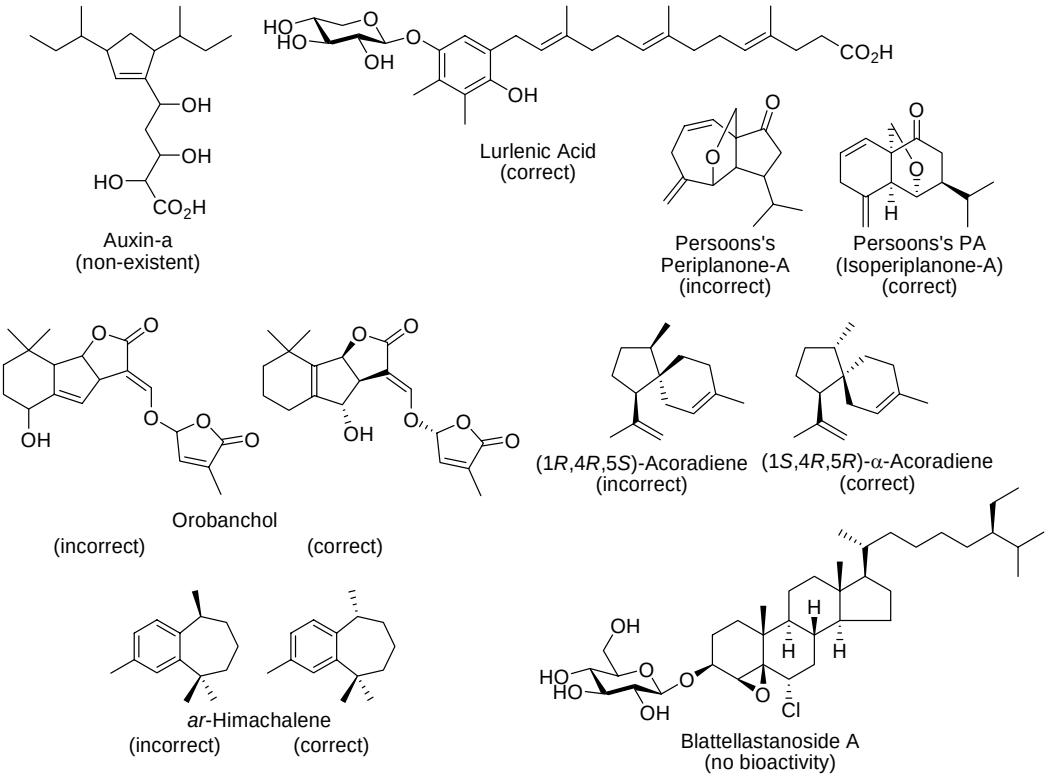
Many incorrect structures of biomolecules have been proposed for natural products. Synthesis of compounds having the proposed structures often enabled us to judge the correctness of the proposals. In some cases, we were able to revise the structures by synthesizing the biomolecules themselves. In other cases, we were able to definitely disprove the proposed structures.

Some examples given in this lecture include the following compounds as shown below [1].



Reference:

[1] Mori, K. *The Chemical Record* **2005**, *5*, 1-16.

NEW APPROACHES TO POTENTIALLY BIOACTIVE SMALL PEPTIDES AND PEPTIDE MIMETICS

Armin de Meijere, **Michael Limbach, Farina Brackmann, Boris Zlatopolskiy, Oleg Larionov, Alessandra Zanobini and Alberto Brandi**

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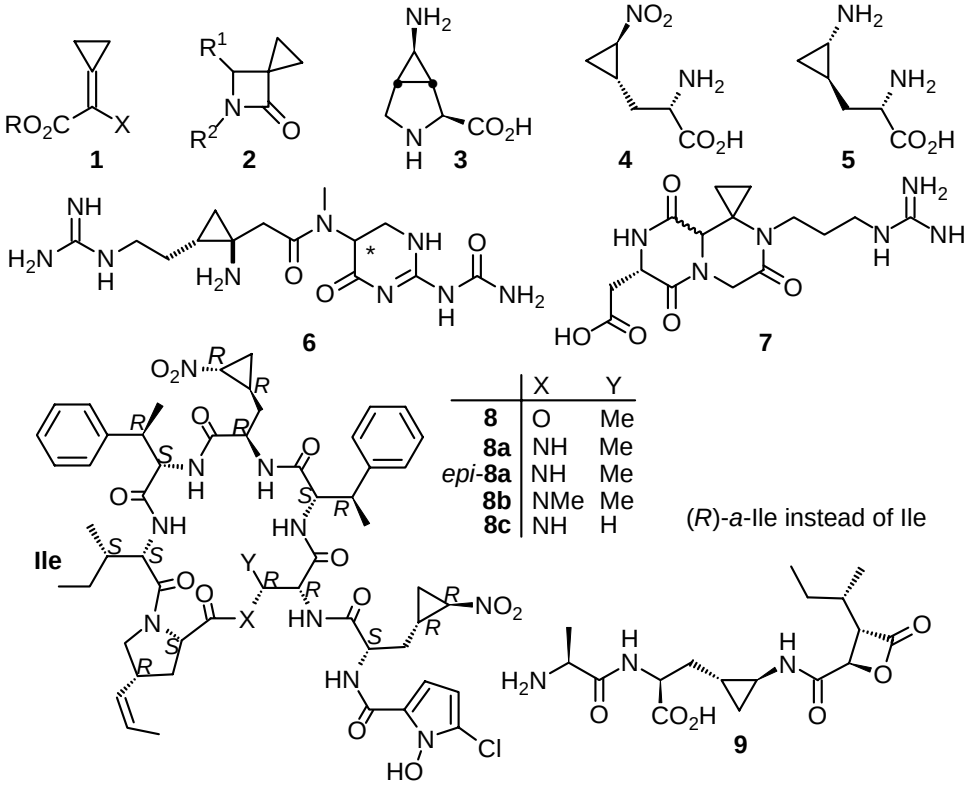
The facile accesses to some versatile building blocks such as **1**-X [1] and **2**–**5** [2] will be presented. Applications of **1**-X towards the synthesis of RGD mimics of type **6** and analogues **7** of the naturally occurring potent antiinfective TAN 1057 will be discussed.



The use of the spirocyclopropanated β-lactame **2** and the proline analogue **3** in the building of small peptide analogues will be demonstrated. (Nitrocyclopropyl)alanine (**4**) and (aminocyclopropyl)alanine (**5**) served as essential components in the total syntheses of the bioactive peptidolactones Hormaomycin **8** [3] with analogues and Belactosin A [4] **9**. These syntheses and some aspects of structure-activity relationships of these compounds will also be presented.

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ENT-STEROIDS: CHEMISTRY AND BIOLOGY

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Steroids are produced by the enzyme-mediated epoxidation and cyclization of squalene to lanosterol. This process produces only one enantiomer of lanosterol and hence steroids derived from lanosterol have the same 'natural' absolute configuration. *Ent*-steroids are the non-naturally occurring enantiomers (mirror images) of natural steroids. Early studies of *ent*-steroids focused largely on their hormonal actions, and it was found that *ent*-steroids lacked hormonal activity. Perhaps for this reason interest in *ent*-steroids waned, and until recently, there has been little sustained interest in either their chemistry or biology. However, not all the biological actions of steroids are explained by their binding to steroid receptors. There are instances in which the physical properties of the steroid may be of even greater importance (e.g. consider the structural effects of cholesterol on cellular membrane properties). Indeed it may be necessary to distinguish between the receptor and non-receptor mediated actions of steroids in order to understand fully steroid mechanisms of action. Because an *ent*-steroid has the mirror image shape of its corresponding natural steroid, it is expected to have different receptor-mediated actions. By contrast, because the *ent*-steroid is an enantiomer of the natural steroid, it has the same physical properties as the natural steroid. Thus, steroid actions caused by the physical properties of the natural and *ent*-steroid in a non-chiral or pseudo non-chiral environment (e.g., lipid membrane) will be the same. Hence, enantioselectivity can be used to distinguish between the receptor and non-receptor mediated mechanisms of steroid action. This lecture will discuss methods useful for the synthesis of *ent*-steroids and examples from our studies on their biological actions.

CATALYTIC ASYMMETRIC SYNTHESIS OF BIOLOGICALLY SIGNIFICANT NATURAL PRODUCTS

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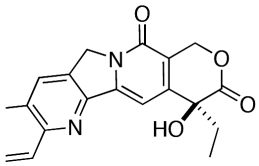
Development of catalytic asymmetric construction of tetrasubstituted carbons such as cyanosilylation of ketones, Strecker reactions of ketoimines and allylation of ketones is discussed. The reactions were successfully applied to the synthesis of optically pure camptothecin **1**, oxybutynin **2**, fostriecin **3**, sorbinil **4** and triazol antifungal agents **5**. Moreover, studies on the catalytic asymmetric total synthesis of garsubellin A **6** will be presented.



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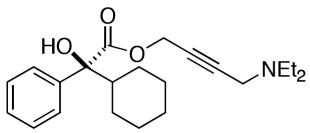

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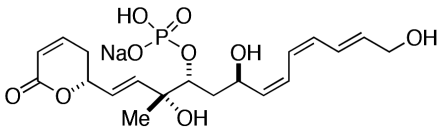

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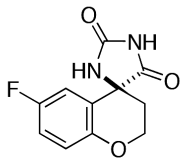

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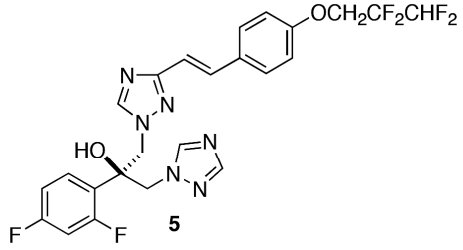

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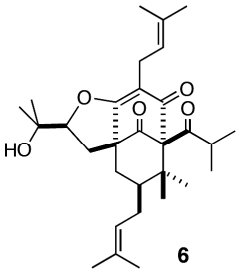

6

LIST OF 30 MINUTE LECTURES

SYNTHESIS OF LINEAR ISOPRENOIDS USING ASYMMETRIC CATALYSIS

Adriaan J. Minnaard, Ben L. Feringa

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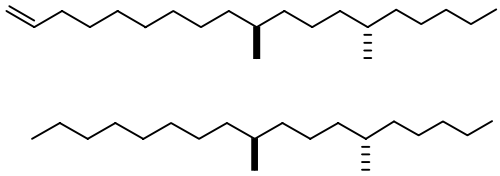
Asymmetric catalysis, and in particular homogeneous transition metal catalysis has become one of the most important ways of making chiral compounds with high enantioselectivities. In this field, the copper-catalyzed asymmetric conjugate addition of organometallic reagents is thoroughly studied as it involves the formation of a carbon-carbon bond and a stereocenter.

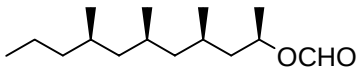
In our group we have successfully developed the asymmetric conjugate addition of dialkylzinc reagents to unsaturated ketones, lactones, alkylidene malonates, and nitro compounds. In our efforts to use this methodology in natural product synthesis we realized that the preparation of linear hydrocarbons containing multiple stereocenters is a challenging field to apply these conjugate addition reactions.

Recently, we have developed a general method to prepare enantiopure saturated isoprenoid building blocks [1]. The method allows the preparation of all four stereoisomers (ee > 99%, de > 98%). To demonstrate the synthetic versatility of this catalytic approach, it was employed in the total synthesis (see Scheme) of two pheromones of the apple leafminer (*Lyonetia prunifoliella*), a pest endemic to the eastern regions of North America [2].

A second application of the methodology is the preparation of enantiopure deoxypropionate building blocks by iterative conjugate addition reactions. This has been used in an efficient synthesis of Lardolure, the aggregation pheromone of the acarid mite, *Lardoglyphus Konoi*.



apple leafminer pheromones



(-)-Lardolure

References:

[1] Van Summeren, R.P.; Reijmer, S.V.; Feringa, B.L.; Minnaard, A.J. *Chem. Comm.* **2005**, 1387 and references cited therein.

[2] For earlier synthesis of these pheromones see: Y. Nakamura and K. Mori, *Eur. J. Org. Chem.*, **2000**, 2745.

JATROPHANE DITERPENES AS INHIBITORS OF MULTIDRUG RESISTANCE

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I–86100 Campobasso, Italy,

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Since ancient time, traditional medicine has been based on the use of drugs in the form of plants and their products. These natural remedies are still used worldwide in the treatment of many diseases and for health care practices. Recent scientific research in the fields of chemistry of natural products and pharmacology has been increasingly addressed to the production of drugs as pure compounds. Most of the new identification of chemicals with well defined biological and pharmacological activities have been isolated and structurally characterized from known medicinal plants.

A very interesting class of compounds is found in the group of the plant-derived diterpene metabolites, e.g taxol, forskolin and ginkgolides.

In the course of our chemical survey of bioactive plant metabolites, a large number of diterpenes have been isolated from *Euphorbia* spp. that showed interesting pharmacological activities. In particular, over fifty new jatrophane and modified jatrophane diterpenoids were extracted, purified and characterized from *E. dendroides, E. peplus, E. characias* and *E. amygdaloides.*

These compounds showed to be potent inhibitors of P–glycoprotein, a membrane protein that confers upon cells the ability to resist lethal doses of certain cytotoxic drugs by pumping them out of the cells, thus resulting in a reduced cytotoxic effect. Indeed, because these compounds were based on a structurally homogeneous skeleton, differing only in the substitution pattern, they have constituted an ideal target for the study of the structure–activity relationships of this new class of Pgp inhibitors. Among the others, two compounds, named euphodendroidin D and pepluanin A, were the most powerful inhibitors of daunomycin–efflux activity within the class of jatrophane diterpenes. Their efficiency was found to be at least two–fold higher than conventional modulator cyclosporin A, thus making both compounds very promising lead to improve drug therapy in multidrug resistant cancer.

THE CHEMISTRY OF AROMATIC PRENYLATION - FROM SUBSTRATE SYNTHESIS TO ENZYMATIC MECHANISMS

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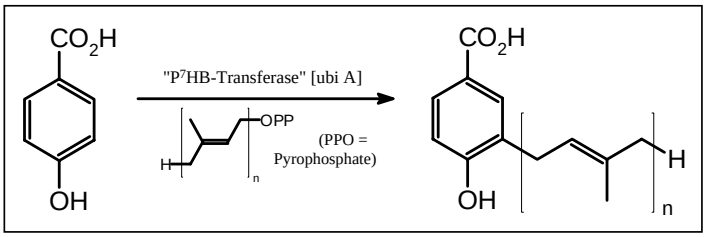
We will present recent improvements in the chemistry and biochemistry of aromatic prenylation, including improved and new syntheses of prenyl diphosphate substrates, mechanism and inhibitor studies of aromatic prenylation, and biocatalytic C-C-coupling reactions.

Aromatic prenylation is important for the biocatalytic access to a multitude of natural products, including so famous members like vitamin E, the phytohormone 8-prenylnaringenin, the dye and drug shikonin, or the hop bitter acids in beer. While hydroquinones are easily prenylated, prenylated benzoic acids are more difficult to obtain by classical methods in despite of their simple structure. 4-Hydroxybenzoate oligoprenyltransferase, in *E. coli* encoded by the gene *ubiA*, is an important key enzyme in the biosynthesis of ubiquinones, itself an essential electron carrier in the respiratory chain of procaryotic and eucaryotic organisms. The two substrate enzyme catalyzes the prenylation of *p*-hydroxybenzoate in the 3-position using oligoprenyl-diphosphates.



The membrane bound *ubiA*-prenyltransferase requires no cofactors. Important parameters and factors such as ease of catalyst preparation and product isolation, enzyme stability, inhibitors, solvent influence, scale-up and conversion boosters will be addressed. A QSAR-study for both substrates as well as the first structural model of an aromatic prenyltransferase, based on homology calculations, will be presented. This is supplemented by mechanistic calculations clarifying the enzymatic mechanism as well as substrate specificity. Future aspects of this research will be discussed briefly.

References: *Aromatic Prenylation:*

[1] Wessjohann, L., and Sontag, B. (1996) *Angew. Chem. Int. Ed. Engl.* **35**, 1697-1699, *Angew. Chem.* **108**, 1821-1823

[2] Wessjohann, L. A., Sontag, B., and Dessoy, M. A. (1999) *Enzymatic C-C Coupling: The Development of Aromatic Prenylation for Organic Synthesis*. Bioorganic Chemistry Highlights and New Aspects (Diederichsen, U., Lindhorst, T. K., Westermann, B., and Wessjonann, L. A., Eds.), Wiley-VCH, Weinheim

[3] S. Zakharova, M. Fulhorst, L. Łuczak, L. Wessjohann, *Arkivoc* **2004**, 79 - 96.

[4] L. Bräuer, W. Brandt, L. A. Wessjohann, *J. Mol. Model.* **2004**, *10*, 317-327.

Related work:

[5] U. Galm, M.A. Dessoy, J. Schmidt, L. A. Wessjohann, Lutz Heide *Chem. & Biol.* **2004**, *11 (2)*, 173 – 183.

[6] W. Brandt, M. A. Dessoy, M. Fulhorst, W. Gao, M. H. Zenk, L. A. Wessjohann, *ChemBioChem* **2004**, *5*, 311-323

[7] W. Gao, R. Loeser, M. Raschke, M. A. Dessoy, M. Fulhorst, H. Alpermann, L. A. Wessjohann, M. H. Zenk, *Angew. Chem.* **2002**, *114*, 2716-2719, *Angew. Chem. Int. Ed.* **2002**, *41*, 2604-2607

ANTICANCER PROPERTIES OF NATURAL BRASSINOSTEROIDS

M. Strnad[1], J. Swaczynová[1], L. Kohout[2], J. Malíková[3], A. Hlobílková[3], Z. Kolář[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany ASCR & Palacký University, CZ-783 71 Olomouc, Czech Republic,

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[2]Department of Steroids, Institute of Organic Chemistry and Biochemistry ASCR, CZ-160 00 Prague, Czech Republic; [3]Institute of Pathology, Medical Faculty of the Palacký University, CZ-775 15 Olomouc, Czech Republic

Brassinosteroids (BRs) are potent plant-growth promoting steroids. The importance of their biological properties led several laboratories to the synthesis of these substances and to the investigation of structure-activity relationships. Antiproliferative and cytotoxic activity of natural brassinosteroids and their structural derivatives on the range of human and animal cell lines have been tested. We have been using the following cell lines: *HELA* (human cervical carcinoma), *MCF7* (human breast adenocarcinoma), *NIH 3T3* (mouse fibroblasts), *HOS* (human osteogenic sarcoma), *HL 60* (human promyelocytic leukemia), *G 361* (human malignant melanoma), *K562* (human erythroleukaemia), *CEM* (human lymphoblastoid leukaemia), U-266 and ARH-77 (human myeloma). Tested drugs were added to the cell cultures in six different concentrations and kept at 37^{o}C and 5% CO_2 for three days. After that, viability of cells in cultures was examined by calcein assay. The concentration killing 50% of tumour cells was calculated. Our research focused on the primary mechanism of action of plant hormones brassinosteroids in cell division cycle has showed that natural plant BRs are rather non-specific inhibitor of cell division. Among 20 BRs tested only 28-homobrassinolide and 24-epicastasterone exhibited interesting anticancer activities. Surprisingly, among BR derivatives, we have discovered some compounds which specifically inhibit cell division of selected cancer cells at micromolar concentration. The tested BRs also causes cell cycle blocks at G2/M transition. Anti-estrogenicity assay showed that all the compounds retain relatively strong antiestrogenic activity in relation to 2-methoxyestradiol used as a standard compound. These compounds also induce apoptosis of different cancer cells *in vitro*. The details of mechanisms of anticancer activities of brassinosteroids will be given during oral presentation.

This work is supported via grant MSM 6198959216.

UNUSUAL TERPENOIDS FROM AFRICAN MEDICINAL PLANTS

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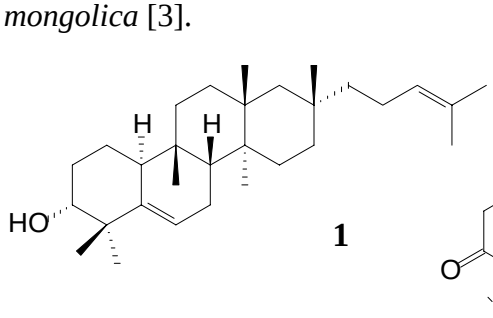
Investigations into the phytochemistry of many African plant species has led to the isolation of many unusual terpenoids and terpenoid-derived compounds. Examples of compounds isolated from the Gentianaceae, Rutaceae (Ptaeroxylaceae) and Meliaceae families will be discussed.

Four novel triterpenoids, **1 – 4**, have been isolated from the stem bark of *Anthocleista grandiflora* Gilg which is a large forest tree of the eastern and southern African tropics and the Comores. *Anthocleista* is a small genus of only fourteen species, eleven of which occur on continental Africa and three are endemic to Madagascar [1]. In southern Africa, the tree is traditionally used to treat malaria [2]. The skeleton of **1** has not been reported previously and **2-4** are the C-13β-methyl isomer of the bauerane class of triterpenoids. This type of skeleton has been reported once previously in a compound from *Artemisia mongolica* [3].



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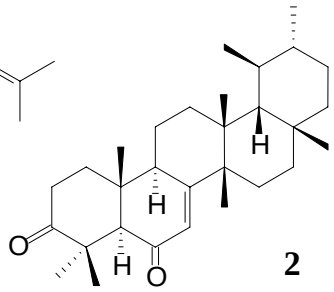

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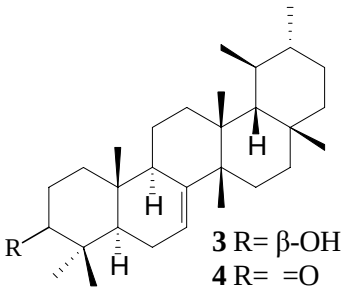
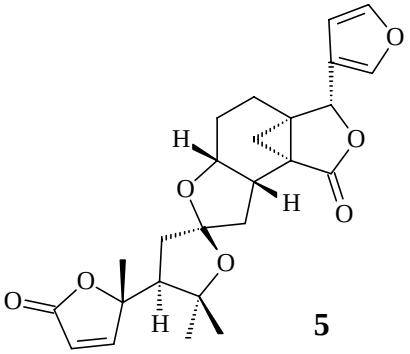


3 R= β-OH
4 R= =O

The isolation of a range of compounds, such as, the limonoid-derived, **5**, and quassinoid, **6**, from the Ptaeroxylaceae has given confirmation of the taxonomic positioning of this genus within the Rutaceae and will be discussed.



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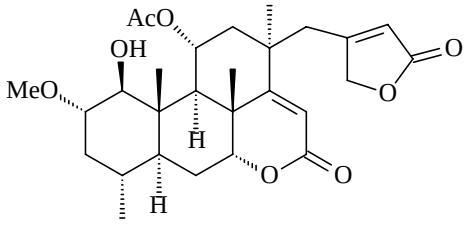

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References:

[1] Leeuwenberg, A.J.M. 1992: *Anthocleista grandiflora*. Flowering Plants of Africa 52: t. 2080.

[2] Palmer, E. and Pitman, N. 1972: Trees of southern Africa. Volume 3. A.A. Balkema, Cape Town: 1845-1847.

[3] Hu, J.-F., He. W-Y., Kong, M., Jia, Z.-J. & Feng, X. 2000: Mangolenin: A new triterpene from *Artemisia mongolica.* Natural Product Letters 14:211-215.

BIAŁOWIEŻA PRIMEVAL FOREST- NATURE TREASURE OF POLAND

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Vast plains of Europe have been intensively managed by humans since ages. This is why in the European temperate zone only very few patches of old-growth woodlands remained, which once covered much of the continent. One of these remnants is the Białowieża Forest, still harboring an outstanding biodiversity. It provides shelter for many rare and endangered species, hardly seen in the wild in other parts of Central and Western Europe, such as wolves, lynx, and last but not least, European bison. The Białowieża forest covers 1500 square kilometers, almost in the middle of otherwise largely deforested East-European plains, at the verge of the watershed of the Black and Baltic Seas.

The history of Bialowieża tree stands dates back to the end of the last glaciation, that is 12,000 before present. At first, large areas were covered mostly by taiga-like vegetation with birches, aspens and pines as dominant species. About 5,000 years ago, with a warming of climate, mixed deciduous forest developed here, very much alike this, that can be seen in Bialowieża today. This was also the time, when humans began to explore the forest, leaving first, still visible traces of their presence. However, this was not until XX century, when human activity begun to severely affect once vast tree stands of Bialowieża. Today, the very last patch of the old-growth never affected by logging, covering just 47 square kilometers, consists the core area of the Białowieża Forest, that is, the Strict Reserve of the Białowieża National Park.

For many centuries the Bialowieża Forest was the hunting ground of Polish kings, and later Russian tsars. In the XV century Polish kings from Jagiellonian dynasty enforced a deliberate protection of the forest—an unprecedented legal act of their times. The main reason for enforcing strict rules for the protection was to provide food and shelter for population of the main game- European bison. The system of protection had worked well until the World War I, which brought a tremendous devastation of the forest due to logging as well as a total demise of the population of European bison. Successful efforts leading to the restitution of the European bison started already in 1919. Thanks to unprecedented dedication of a very few researchers, initially small herd of merely 54 individuals collected from zoos and safari parks all over Europe increased in size over the years. Today, almost 600 animals roam freely through the Białowieża forest. There is no doubt that historic protection of European bison was a key factor in conservation of the Białowieża Forest. Hopefully, its beauty and biological diversification will continue to attract the attention of scientists and the admiration of visitors for the centuries to come.

STEROLS OF HARMFUL MARINE ALGAE: SYNTHESIS AND METABOLISM

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The harmful algae that produce toxins such as saxitoxin and the brevetoxins also often contain sterols with unusual structures. We recently hypothesized that these sterols serve as chemical defenses by interfering with the nutrition and growth of marine invertebrates [1]. These sterols may be refractory to the normal bioconversion of dietary sterols to cholesterol, and may also interfere with the biosynthesis of steroid hormones by mechanism-based inhibition. To test this, methods for the synthesis of gram quantities of algal sterols were developed and used to prepare specifically ^{13}C-labeled material. This was incorporated into the diet of juvenile bay scallops (*Argopecten irradiens*) and brine shrimp (*Artemia salina*). Analysis by ^{13}C-NMR spectrometry indicated the metabolic fates of the sterols. Addition of a sterol bearing label in a different position was used as a positive internal control in cases where no bioconversion of the test sterol was detected.

Acknowledgements: This work was made possible by ECOHAB and NOAA Coastal Oceans Programs.

References:

[1] Giner, J.-L.; Faraldos, J. A.; Boyer, G. L., "Unique Sterols of the Toxic Dinoflagellate *Karenia brevis* (Dinophyceae): A Defensive Function for Unusual Marine Sterols?" *J. Phycol.* **2003**, *39*, 315-319.

MEROTERPENOIDS FROM MUSHROOMS, A COLOURFUL GROUP OF NATURAL PRODUCTS

Wolfgang Steglich, Jürgen Beyer, Nicole Feling, Barbara Koch, Martin Lang, Susanne, Lang-Fugmann, Bernd Sontag, Peter Spiteller, and Matthias Rüth

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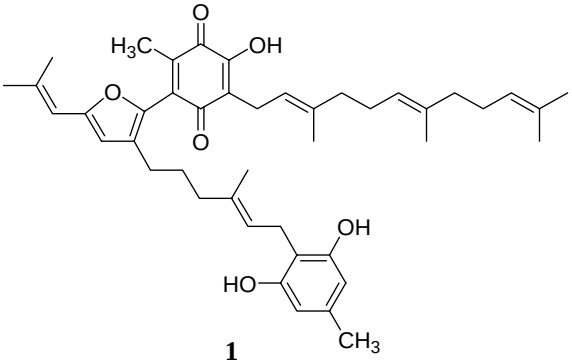
Mushrooms produce a variety of metabolites in which an aromatic nucleus is substituted by a terpenoid chain (meroterpenoids). Some of these compounds have rather unique structures, e.g. the blue albatrellin A (**1**) from *Albatrellus flettii* and the red tridentoquinone (**2**) from *Suillus tridentinus*. **1** was synthesized by coupling of two monomeric co-metabolites, mimicking its possible biosynthesis.



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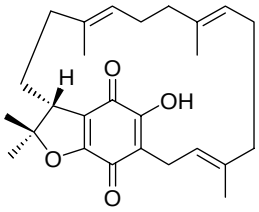

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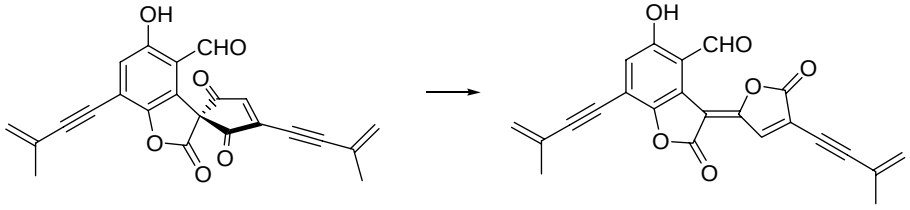
The spirolactonedione ochroleucin A_1 (**3**) is responsible for the red colour reaction exhibited by the yellowish stem base of *Russula ochroleuca* on treatment with aqueous KOH. The compound shows interesting spectroscopic properties and rearranges easily into the more stable dilactone ochroleucin A_2 (**4**). Biosynthetic studies reveal that **3** is formed from two monomeric species by oxidative dimerization.



3 **4**

Some edible boletes produce meroterpenoids, e.g. **2**, originating from 3,4-dihydroxybenzoic acid. The results of recent studies on the biosynthesis of these compounds by feeding ^{13}C-labeled precursors [1] to young fruit bodies will be presented [2].

References:

[1] M. Lang, W. Steglich, *Synthesis* **2005**, 1019.
[2] A. Mühlbauer, J. Beyer, W. Steglich, *Tetrahedron Lett*. **1998**, *39*, 5167.

RECENT DEVELOPMENT OF THE CYCLOPROPANOL METHODOLOGY FOR THE CONSTRUCTION OF METHYL OR METHYLENE BRANCHED ACYCLIC COMPOUNDS

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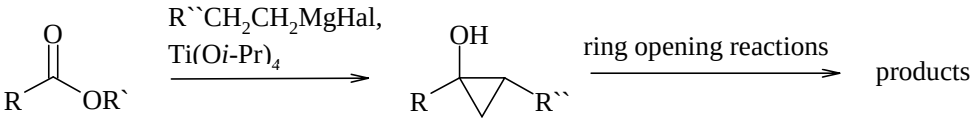
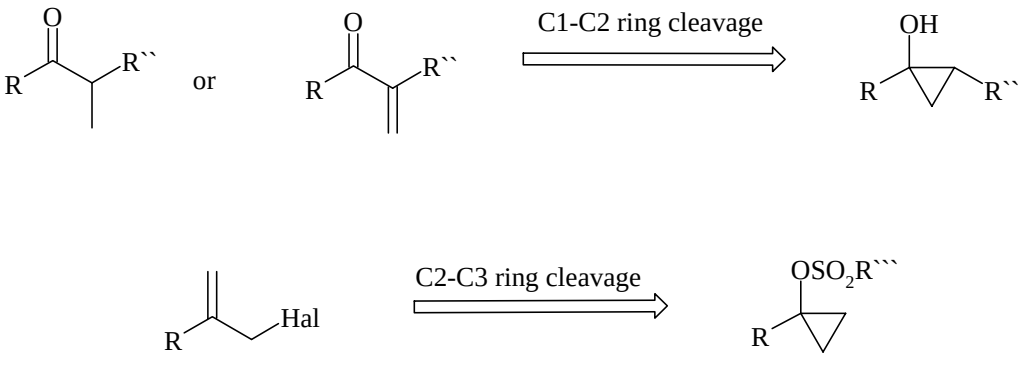
The reaction of carboxylic esters with the Grignard reagents in the presence of titanium(IV) alkoxides leads to double alkylation of ester carbonyl and formation of 1-substituted cyclopropanols. Due to usually high yields and latent multifunctionality of the cyclopropanols, this reaction possesses considerable synthetic potential and attracts our interest over the past few years.



Regioselective ring-opening reactions of substituted cyclopropanols allow to prepare various saturated, unsaturated and α,β-epoxy ketones, allyl halides, 1,3-dienes, 5-substituted isoxazoles and other functional compounds. Some of these transformations we have explored for the construction of acyclic compounds with methyl or methylene branched skeletons starting from linear precursors. The methodology is based on the preparation of the key synthetic intermediates by reactions of C1-C2 or C2-C3 bonds cleavage of substituted cyclopropanols or their sulfonates with the formation of the corresponding branched ketones or 2-substituted allyl halides.



The data on the application of the cyclopropanol methodology to the syntheses of some chiral natural products will be presented.

CATALYTIC ASYMMETRIC SYNTHESIS OF ISOPRENOIDS: GERMACRADIENES AND LIMONOIDS

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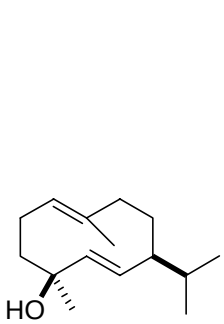
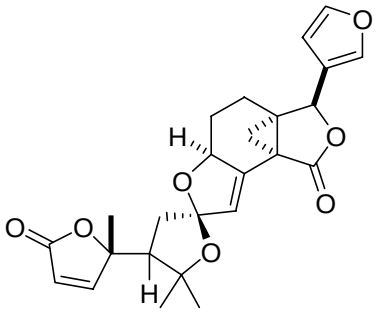
The pine sawfly *Neodiprion sertifer* is a common pest for pine trees of the northern hemisphere. The population of larvae can cause extensive defoliation, resulting in serious economical damage. Therefore, great effort has recently been directed in trying to monitor and control the population of sawfly species. It has been shown that the resin secreted by pines interferes with the development of larvae. The main component of the sesquiterpene fraction of the resin of *Pinus sylvestris* is 1,6-germacradien-5-ol, first isolated by Bohlmann and co-workers from *Senecio phonolithicus*.

Cneorin C was originally isolated from the xerophytic shrub *Cneorum pulverulentum*, native to the Canary Islands, in the late 1970's. This shrub hosts a variety of bitter principles called cneorins, all of which contain the [4.3.1]propellane ring system. Biogenetically these compounds are thought to be related to the limonoid triterpenes. Due to a lack of material from natural sources, these compounds have not received proper pharmacological screening. In addition to *Cneorum pulverulentum*, the *Cneoraceae* plant family consists of two other species, *Cneorum tricoccon*, which is native to coastal areas of the western Mediterranean, and *Cneorum trimerum*, which belongs to the flora of Cuba. The tricoccins were isolated from the former, but the latter has not been adequately studied due to a lack of access to the required plant materials. Recently, some close structural relatives of the cneorins, the cedkathryns, were isolated from *Cedrelopsis gracilis* from Madagascar. In addition, some compounds that could result from rearrangements of the cneorins or the tricoccins, for example cedmilinol, have been isolated from *Cedrelopsis grevei*.



1,6-Germacradien-5-ol



Cneorin C

This lecture will present our results on the synthesis of the title compounds utilising enantioselective metal-catalysed reactions.

STEROID SAPOGENINS STILL A SOURCE OF NEW REACTIONS AND NEW COMPOUNDS

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Steroid sapogenins have a long history, in the past century this family of compounds was protagonist in the discovery of new reactions and in the development of synthetic applications [1]. We have recently reported the preparation of some bioactive spirostanic analogues of brassinoesteorids and found that the presence of an oxygen atom at C-23 produces and increase in the plant growth promoting activity, (Figure 1) [2].

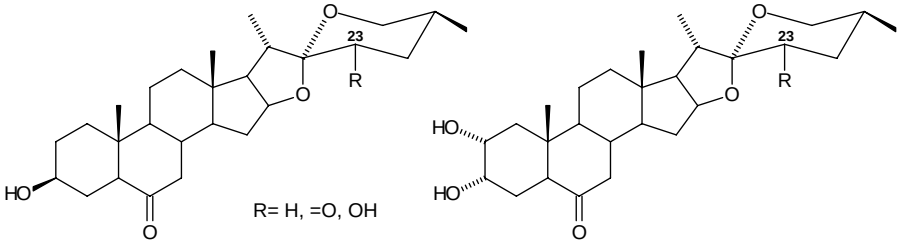
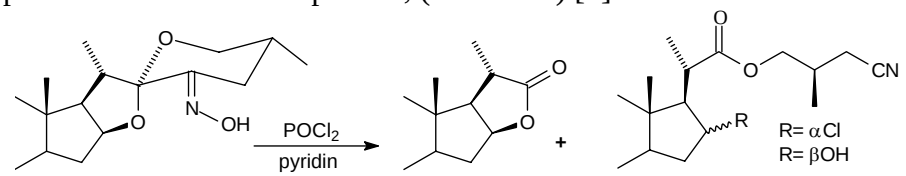


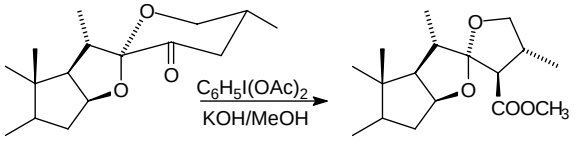
Figure 1

More recently, new reactions involving the spiroketal side chain are being reported, showing that steroid sapogenins still an interesting target for further investigations. In particular compounds and reactions derived form the presence of functionality at C-23 may be of synthetic utility. We have described that the Beckmann rearrangement of 23-hydroxyiminodiosgenin acetate follows the so-called abnormal course to produce mixture of compounds, (Scheme 1) [3].



Scheme 1

Applications of hypervalent iodine to organic synthesis have received considerable attention. In particular, the reaction of iodosobenzene with steroidal ketones has been reported to follow different courses depending on the steric hindrance. We have studied the reaction of 23-oxosapogenins with diacetoxyiodobenzene in KOH/MeOH and found that it follows the course Favorskii rearrangement which results on F-ring, (Scheme 2). The reaction proceeds in such a way in which the configuration of the starting 23-oxosapogenin at C-25 is transferred to the new quiral center generated at C-23.



Scheme 2.

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SALVINORIN A: BIOLOGICALLY UNIQUE DITERPENOID FROM *SALVIA DIVINORUM*

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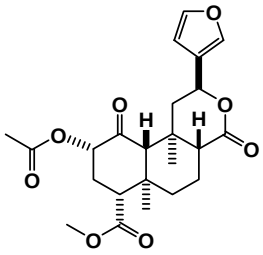
Salvia divinorum (Epling and Jativa) is a psychoactive plant that has been used for centuries by the Mazatecan people of Mexico for illness and divination. The major secondary metabolite and hallucinogenic principal, salvinorin A, is a neoclerodane diterpenoid and has demonstrated high affinity and exclusive selectivity for the κ-opioid receptor (KOR) as an agonist [1,2]. The KOR has been the topic for research aimed at overdose/addiction safe antinociceptive therapies, but perhaps overlooked for its ability to alter perception on a more general, all-inclusive level. As a potent and selective κ-opioid agonist, salvinorin A provides a new model for research aimed at pain, opiate addiction, and dementia management. This presentation will discuss the mechanism of action, the semi-synthetic modifications of salvinorin A, and the biological evaluation of its derivatives.



Acknowledgments: National Institutes of Health Grant RO1DA017204 and the National Institute of Mental Health, Psychoactive Drug Screening Program.

References:

[1] Ortega, A. et al. (1982) *J. Chem. Soc., Perkin Trans. I*, 2505-2508.
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RECENT DEVELOPMENTS IN PALLADIUM-CATALYSED CARBONYLATION OF STEROIDS — AN ALTERNATIVE APPROACH TO CARBOXYLIC ACID DERIVATIVES

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After a great progress in the past few decades, organo-transition metal chemistry has become a very powerful tool in organic synthesis offering the most efficient solutions to practical problems in many cases. The enhanced selectivities, well-defined mechanism, and the applicability of standard techniques are the main features, which make the homogeneous catalytic reactions attractive also in the synthesis of steroids.

There is an increasing interest in developing new strategies to introduce functional groups into specific positions of steroidal nuclei in order to modify their biological properties. Transition metal catalyzed reactions have proved to be versatile tools both for the construction of the steroid framework from easily available building blocks and for the functionalization of the steroidal skeleton [1].

By palladium-catalysed carbonylation, carbon monoxide can be introduced directly into a number of different sites in an organic molecule leading to the synthesis of aldehydes, ketones, carboxylic acids and their derivatives, lactones, lactames, etc. The products can often be obtained in good yield and with high selectivity usually under very mild conditions. In addition, palladium-catalysed carbonylation is compatible with many functional groups, and therefore, more advantageous than conventional methods.

Besides, in these reactions either enol-triflates or vinyl iodides, both of which can be easily synthesised from the readily available keto derivatives, can be used as starting material. Several publications and patents show the effectiveness of carbonylation for the synthesis of derivatives of biological importance (*e.g.* a number of 5α-reductase inhibitors).

In the present paper the most important achievements in carbonylation of steroidal substrates will be reviewed together with a more detailed discussion of our own results obtained in this field.

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[1] Skoda-Földes, R.; Kollár, L. *Chem. Rev.* **2003**, *103*, 4095-4129.

CROSS-COUPLING REACTIONS FOR STEROID MODIFICATION: FROM ARYLATION TO MACROCYCLES SYNTHESES.

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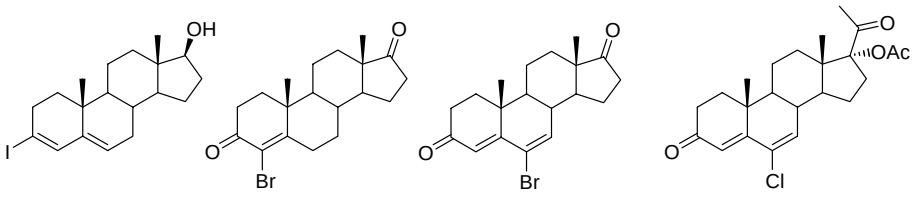
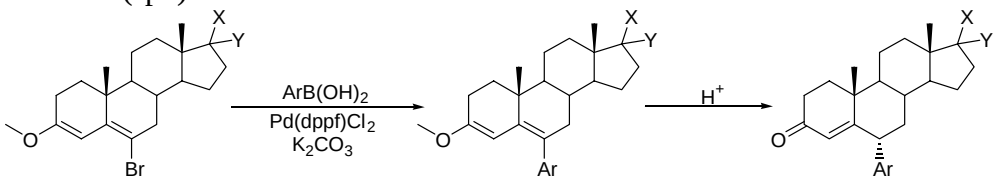
The Pd- and Ni-catalyzed coupling of vinyl (aryl) halides and nucleophilic compounds is a powerful approach to carbon-carbon and carbon-heteroatom bond formation. Earlier several steroid triflates and iodides were used for Pd- catalyzed Heck and Sonogashira reactions, and for cross-coupling with organometallic compounds [1]. During our research on the synthesis of new aromatase inhibitors we have found that not only iodosteroid **1**, but also bromosteroids **2, 3** and even inert chloromadinone **4** can be successfully used in the Suzuki-Miyaura arylation. This approach provides high yields of new 3-, 4-, 6- arylsteroids with different functional groups in the aryl substituent.



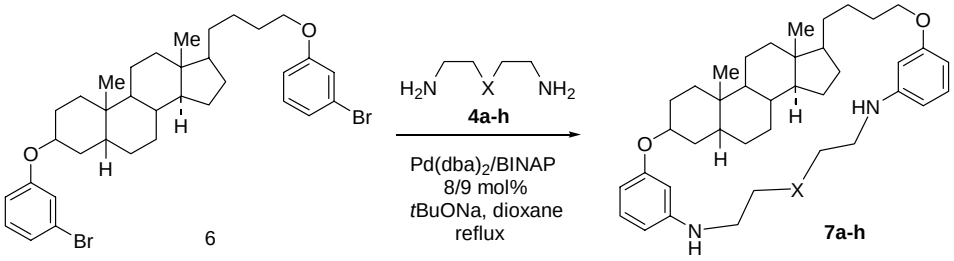
1 **2** **3** **4**

We have shown that steroid enol bromides **5** can be used for the attachment of Ar-groups to the 6-(sp3)-carbon atom of steroid skeleton.



5 X,Y = O; X = Ac, Y= OAc

Macrocycles containing steroid moieties attract a keen attention of chemists due to their unique properties as selective artificial receptors and sensors, as architectural components in biomimetic/molecular recognition chemistry [2]. We have elaborated a new approach to the macrocycles containing steroid fragments which is based on the palladium-catalyzed amination of aryl halides, for example:



6 **7a-h**

X =CH_2NHCH_2 (**a**); $NH(CH_2)_2NH$ (**b**); $NH(CH_2)_3NH$ (**c**); $CH_2NH(CH_2)_2NHCH_2$ (**d**); $CH_2NH(CH_2)_3NHCH_2$ (**e**); $NH(CH_2)_2NH(CH_2)_2NH$ (**f**); $O(CH_2)_2O$ (**g**); $CH_2O(CH_2)_2O(CH_2)_2OCH_2$ (**h**).

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BIOSYNTHESIS OF BRASSINOSTEROIDS IN RYE VIA 2,3-EPOXY INTERMEDIATES

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2,3-Epoxybrassinosteroids such as secasterone and 24-*epi*-secasterone previously have been reported in seeds of *Secale cereale* [1;2] and *Lychnis viscaria* [3], respectively. In rye seedlings (*Secale cereale*), the biosynthesis of secasterone and 2,3-diepisecasterone was shown to start from teasterone and typhasterol and proceed through secasterol [2].

A series of feeding experiment was undertaken in order to investigate whether the epoxybrassinosteroids are final biosynthetic products or undergo further conversion [4]. [26-$^2H_3$]Secasterone, [26-$^2H_3$]2,3-diepisecasterone, [26-$^2H_3$]castasterone, and [26-$^2H_3$]epicastasterone [5] were administered hydroponically to rye seedlings and biosynthetic products analyzed by an improved LC-MS-SIM method [6].

The results showed that secasterone, by opening the epoxide, was converted to 2-epicastasterone as the major product and 3-epicastasterone and castasterone as minor metabolites in rye seedlings. 2-Epicastasterone was found as the only product of 2,3-diepisecasterone. Inversion of configuration of 2β to 2α and 3β to 3α was found, *i.e.* castasterone was detected as a product upon administration of 2-epicastasterone and 3-epicastasterone, respectively, to rye seedlings.

Together with recent results on secasterone biosynthesis [2], this study provides evidence for a new biosynthetic networking in the pathway to castasterone in *Secale cereale*. These findings demonstrate that the biosynthetic sequence teasterone/typhasterol → secasterol → secasterone → 2-epicastasterone/3-epicastasterone → castasterone is operative in this plant [4]. This route represents an alternative to the direct C-2α-hydroxylation of typhasterol to castasterone in the established early C-6 oxidation pathway.

References:

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[5] Khripach, V.A., Zhabinskii, V.N., Konstantinova, O.V., Antonchick, A.P., Schneider, B. (2002) Steroids 67, 587-595.

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ANALYSIS OF BRASSINOSTEROIDS

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The identification of brassinosteroids (BS) in plant raw material is a complex and multistep procedure including many operations. Because of the very low BS content in plants, it is not always possible to isolate them in quantities that are sufficient for the recording of all spectral characteristics. In such cases the identification of BS has to be done by making derivatives and comparing these with separately synthesized authentic samples. Gas chromatography in combination with mass spectrometry (GC-MS) in different modifications is employed very often for the identification of natural BS. Usually, moderately volatile methaneboronic acid derivatives of BS are used for spectroscopic analysis. An alternative for GC-MS is a method based on high-performance liquid chromatography of BS derivatives suitable for UV-, or other methods of detection. These methods in different modifications were widely used and allowed identification of a number of naturally occurring BS, including three new hormones recently discovered by joint research group of our laboratory and Max Planck Institute for Chemical Ecology. Unfortunately, all these approaches are rather expensive and time consuming, and that is why they are not suitable for the routine analysis of samples. At the same time, extensive research programs on BS biosynthesis, their species-specific distribution in plants and mechanism of action, initiated nowadays in many laboratories, need newly elaborated techniques which allow more sensitive, speedy and cheaper BS analysis. It is also true in connection with permanently expanding agricultural use of BS, where deep knowledge on BS dynamic in plants, their metabolism in animals and related problems is necessary.

The immunoassay technique, using antibodies against BS as an analytical instrument, looks the most convenient for this purpose. Although immunoassays for analysis of different plant hormones have been developed and they are readily available for plant physiologists, there are seemingly no efficient immunochemical assay systems for BS. In this lecture, our efforts toward elaboration of immunoenzymatic assay for brassinosteroids will be described.

DIMOLYBDENUM METHOD FOR DETERMINATION OF THE ABSOLUTE CONFIGURATION OF *vic*-DIOLS – FOUNDATIONS AND DVELOPMENTS

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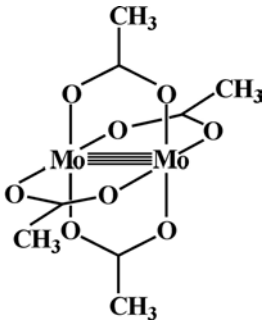
The determination of the absolute configuration of *vic*-diols has received an increasing attention recently, mainly due to their common occurrence in nature both in a free form as well as of their respective esters or amides. In order to successfully apply the circular dichroism spectroscopy (CD) for this particular purpose a *vic*-diol unit should be transformed into the so-called cottonogenic derivative, i.e., derivative displaying appropriate absorption characteristics. One of the proposed methods consists of generation of chiral complexes *in situ* by mixing the chiral but non-absorbing *vic*-diol with an achiral transition metal complex acting as an auxiliary chromophore. It has been previously shown that the dimolybdenum tetraacetate [$Mo_2(OAc)_4$] is one of such agents which can form optically active complexes with diols. The CD spectra of resulted complexes can be successfully used for the stereochemical assignment [1].



To-date a variety of different *prim*/*sec* and *sec*/*sec vic*-diols were investigated in the presence of the Mo_2-core. On the basis of the obtained results a helicity rule correlating the sign of the O–C–C–O torsional angle with the sign of the Cotton effect (CE) occurring around 310 nm was established: ″a positive (negative) sign of this torsional angle correlates with a positive (negative) sign of the CE around 310 nm (Figure). The major aim of this work is to study whether the helicity rule, and thereby the dimolybdenum method, can be applied to all types of *vic*-diols. Therefore, in addition to *prim*/*sec* and *sec*/*sec vic*-diols, chiroptical properties of sterically hindered *vic*-diols, i.e., *sec/tert* and *tert/tert*, in the presence of dimolybdenum tetraacetate will be examined.

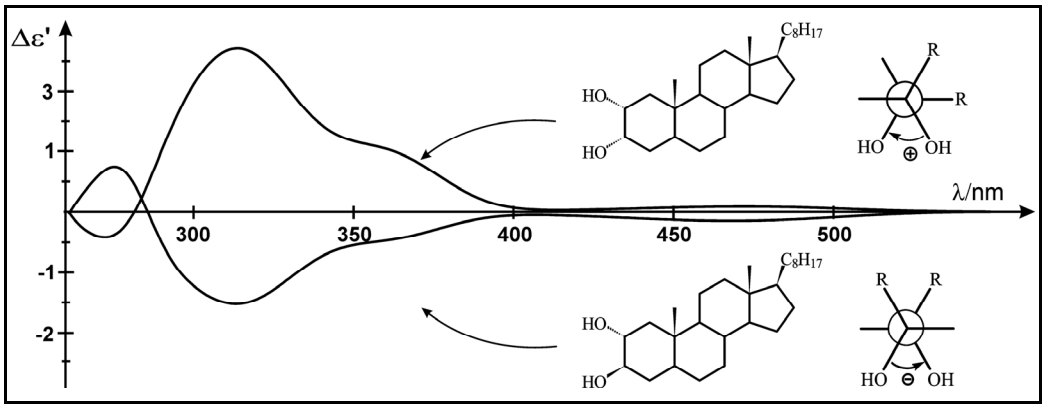


Figure: CD spectra of 2α,3α- and 2α,3β-cholestandiols in the presence of [$Mo_2(OAc)_4$].

References:

[1] Frelek, J.; Klimek, A. Ruśkowska P. *Current Org. Chem*. **2003**, *7*, 1081-1104.

OSW SAPONINS: FACILE SYNTHESIS TOWARD A NEW TYPE OF STRUCTURES WITH POTENT ANTITUMOR ACTIVITIES

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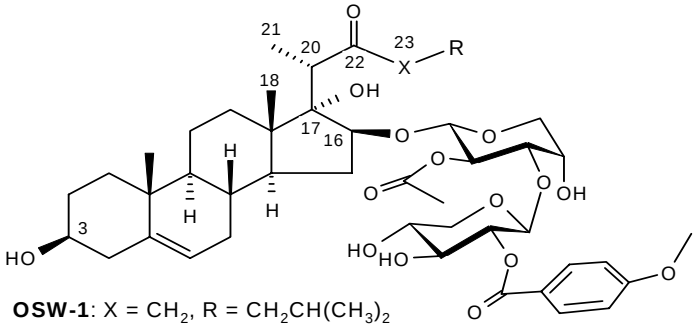
Saponin OSW-1, featuring a novel 16β,17α-dihydroxycholest-22-one aglycone and an acylated disaccharide at the 16-OH, was disclosed by Sashida, Mimaki, and coworkers in 1992 from the bulbs of *Ornithogalum saudersiae*, a pleasant perennial garden plant of the lily family widely cultivated in southern Africa. Tremendous attention has been given to this molecule since the test of its antitumor activities in 1995: while showing little toxicity toward normal cells, OSW-1 inhibited the growth of a variety of malignant tumour cells with 10- to 100-fold potency than those of the clinically applied anticancer agents, such as mitomycin C, adriamycin, cisplatin, camptothecin, and taxol. Continued researches on *O. saudersiae* and its taxonomically related plants have revealed a family of the 16β,17α-dihydroxycholest-22-one saponins. Extensive efforts have also been made toward the synthesis of OSW-1, the first and major component being isolated. The aglycone was first synthesized by Fuchs and Guo in 1998. Shortly, we achieved the coupling of the aglycone with the disaccharide moiety to complete the total synthesis of OSW-1. Recently, the groups of Jin and Morzycki also finished the total synthesis, respectively. However, only a limited number of OSW saponin analogues have been obtained by means of the chemical synthesis for SAR studies. Detailed biological, toxicological, and pharmacokinetic studies still wait for altering the nature of the synthetic challenge from total synthesis to the preparation of a variety of analogues, derivatives, and hopefully a lead in multigram quantities. Recently, we developed a novel and efficient approach to construction of the 16β,17α-dihydroxycholest-22-one structure. Elaborations of this core structure into OSW-1 and especially into its side chain analogs (e.g., **1**-**3**) were straightforward. Some of those analogs were found as potent as OSW-1 to induce the apopotosis of tumor cells.



OSW-1: X = CH_2, R = $CH_2CH(CH_3)_2$
1: X = O, R = $CH_2CH(CH_3)_2$
2: X = O, R = CH_2CH_3
3: X = O, R = $(CH_2)_{11}CH_3$

Acknowledgements: This work was supported by the National Natural Science Foundation of China (20372070, 20321202) and the Chinese Academy of Sciences (KGCX2-SW-209).

TRITERPENE SAPONINS IN *MEDICAGO TRUNCATULA*

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Barrel medic (*Medicago truncatula* Gaertn.) is closely related to alfalfa (*Medicago sativa* L.) and has been chosen as a model legume for functional genomics projects that incorporate profiling of gene expression (transcriptome), protein expression (proteome) and metabolite expression (metabolome) to better understand the biological processes associated with legumes and their interaction with environment. Profiling and identification of large variety of natural products from *M. truncatula,* including saponins, is crucial to metabolomics and functional genomics. Thus, our present research focused on two groups of secondary metabolites of high importance due to their nutritional and environmental significance.

Triterpene saponins from *Medicago truncatula* aerial parts have been separated and their structures have been determined by the extensive use of 1D- and 2D NMR experiments including ^{1}H-^{1}H (DQF-COSY, 1D-TOCSY) and ^{1}H-^{13}C (HSQC, HMBC) spectroscopy along with ESIMS. Fifteen individual compounds were isolated that included seven medicagenic acid and eight zanhic acid glycosides. Additionally two soyasapogenol B and soyasapogenol E glycosides were identified by MS/MS and TLC. Four medicagenic acid glycosides and eight zanhic acid glycosides were reported for the first time. The common feature of *M. truncatula* aerial part saponins is the (1→3) linkage between the two glucose units at C-3 of medicagenic and zanhic acids, which is different from that found in alfalfa (*Medicago sativa*) where this linkage was always (1→2). This may suggest differences in glucosyltransferases between these two *Medicago* species.

Saponins from aerial parts of *Medicago truncatula* cv. Jemalong A-17, *M. truncatula* Gaertn. var *longispina* Urb. and *M. truncatula* Gaertn. var *truncatula* were profiled and quantified using reverse-phase LC with on line photodiode array detector and electrospray-ionisation mass spectrometry (LC/PDA/ESI/MS/MS). The composition of saponin mixture was very similar in three subspecies with three dominant groups to be recognized as zanic acid, medicagenic acid and soyasapogenol glycosides. Relative proportion of these three groups was also similar in the three subspecies: var *longispina* had 49.5%, 48.1% and 2.4%, var *truncatula* 41.5%, 53.4% and 5.1%, Jemalong A-17 42.1%, 56.6% and 1.3% of zanic acid, medicagenic acid and soyasapogenol glycosides, respectively. The Jemalong A-17 had 30% lower total content of saponins as compared to *M. truncatula* var *longispina* and *M. truncatula* var *truncatula*; in relation to the dry matter the var *longispina* contained 0.22%, var *truncatula* 0.22% and Jemalong A-17 0.15% d.m. of saponins.

Acknowledgements: We acknowledge financial support from Polish Ministry of Science (grant no 2PO6A 037 27).

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DESIGN AND SYNTHESIS OF A NEW VITAMIN D SUPERAGONIST

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The current interest in the therapeutic properties of the natural hormone 1α,25-$(OH)_2$-D_3 and its 1α-hydroxyvitamin D_3 analogues results from the ability of these compounds to control abnormal processes by modulating cell differentiation, inhibiting cell proliferation, and regulating apoptosis, a fact that suggests its possible use in the treatment of cancer and other proliferative diseases. Efforts aimed at developing vitamin D analogues with strong cell-differentiating ability and low calcemic action have led to the synthesis of more than 3000 1a-hydroxyvitamin D analogues and some of these are already on the market and in clinical development.

Based on the crystal structures of human VDR bound to its natural ligand1α,25-$(OH)_2$-D_3 and to superagonist KH1060, it was possible to design a new superagonist ligand, namely **A**, which incorporates a tetrahydrofuranic ring at the side chain. We describe here the synthesis and properties of the new superagonist **A** and its diastereoisomer **B**, which behaves like 1α,25-$(OH)_2$-D_3. The crystal structures provide an explanation for the mechanism of superagonist activity and a rational approach to the design of more potent ligands.

Acknowledgements: We are grateful to A. Steinmeyer (Schering AG) for a generous gift of 1α,25$(OH)_2D_3$. We thank the beamline staff at the ESRF BM30 (Grenoble, France) for technical assistance during data collection. The project was supported by a grant from CNRG to the structural genomics platform and was funded in part by the European Commission as SPINE, contract-no QLG2-CT-220-0098 under the RDT programme 'Quality of Life and Management of Living Resources'. We also thank the Spanish MEC (proyect SAF2004-01885) for financial support of the synthetic part. LCR thanks the University of Santiago de Compostela for a predoctoral fellowship.

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2-ALKYLIDENE ANALOGS OF 19-NOR-1α,25-$(OH)_2D_3$: SYNTHESIS AND BIOLOGICAL ACIVITY

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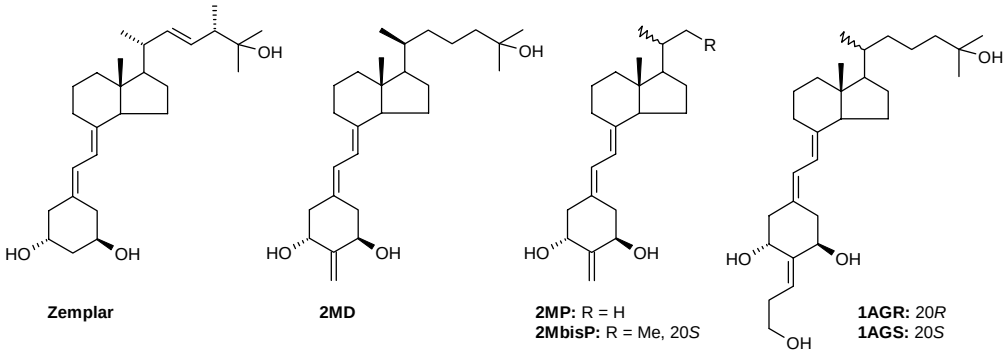
A growing body of biological data indicate that the function of 1α,25-dihydroxyvitamin D_3, [1α,25-$(OH)_2D_3$], extends beyond calcium and phosphorus homeostasis. The vitamin was also found to regulate cellular differentiation and to play a role in immunoregulatory activity. In 1990 we reported a synthesis of the first member of the so-called 19-norvitamins D. This analog, 19-nor-1α,25-dihydroxyvitamin D_3, was characterized by the replacement of the A-ring exocyclic methylene substituent at C-10 by two hydrogen atoms. Biological testing of this compound revealed its selective activity profile with high potency in inducing cellular differentiation and very low calcium mobilizing response. Several similar 19-norvitamins were prepared to date and the most notable among them is 19-nor-1α,25-dihydroxyvitamin D_2 (**Zemplar**), successfully marketed by Abbott for renal osteodystrophy. As a continuation of these studies we synthesized analogs of the natural vitamin D hormone, 1α,25-$(OH)_2D_3$, characterized by transposition of its A-ring exocyclic methylene group from carbon 10 to carbon 2. Among such vitamins, 2-methylene-19-nor-1α,25-$(OH)_2D_3$, possessing unnatural configuration at C-20 (**2MD**), is most remarkable due to its unique ability to induce bone formation. 2-Methylene-substituted 19-norvitamin D compounds with truncated side chains were also prepared and two of them (**2MP** and **2MbisP**) show great promise in the treatment of secondary hyperparathyroidism, cancer and psoriasis. In an effort to further explore the 19-nor class for pharmacologically important vitamin D compounds, the isomeric 2-ethylidene-19-nor-1α,25-$(OH)_2D_3$ compounds were successfully prepared. The promising biological potencies of such analogs, especially those with *E*-geometry of the ethylidene group, encouraged us to further explore this A-ring modification by the synthesis of 2-(3'-hydroxypropylidene)-19-nor-1α,25-$(OH)_2D_3$ analogs. Biological tests revealed that calcemic activity of *E*-geometrical isomers **1AGR** and **1AGS** considerably exceeds that of the native hormone, 1α,25-$(OH)_2D_3$.



Zemplar **2MD** **2MP:** R = H **2MbisP:** R = Me, 20*S* **1AGR:** 20*R* **1AGS:** 20*S*

DIASTEREOMERIC AND GEOMETRIC ANALOGS OF VITAMIN D3 - ENANTIOSELECTIVE SYNTHESIS AND FUNCTIONAL ACTIVITY

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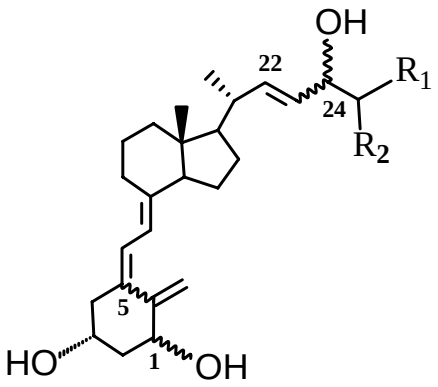
As a part of our search for vitamin D analogs of therapeutic potential we designed and synthesized a series of analogs of 1,25-dihydroxyvitamin D_3 with reversed configuration at C-1 or C-24 and *E* or *Z* geometry of double bond at C-22 in the side-chain or at C-5 in the triene system. The analogs were constructed by a partial synthesis from C-22 heteroaromatic sulfone and the homochiral side-chain hydroxy aldehyde. The most efficient coupling was obtained with C-22 benzothiazolyl sulfone (S. Julia 1991). The highest enantiomeric purity of the side-chain hydroxy aldehyde was obtained using a separation of diastereomeric esters of the respective allyl alcohols. Out of a number of enantioselective syntheses applied the Sharpless asymmetric dihydroxylation of a terminal olefin and Jacobsen catalytic cleavage of terminal epoxide gave significantly lower enantiomeric purity of 64 and 75 %, respectively.



Out of a series of analogs screened on various cancer cell lines (5Z,7Z)-compound was found very active not only on human leukemia (HL-60) and breast cancer (MCF-47) but also on squamous cancer cell line SCC-25 and more than 200-times more active than the parent 1,25-dihydroxycholecalciferol on mitoxanthrone resistant human leukemia line HL60/MX2.

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STEROIDS FROM CARVONE

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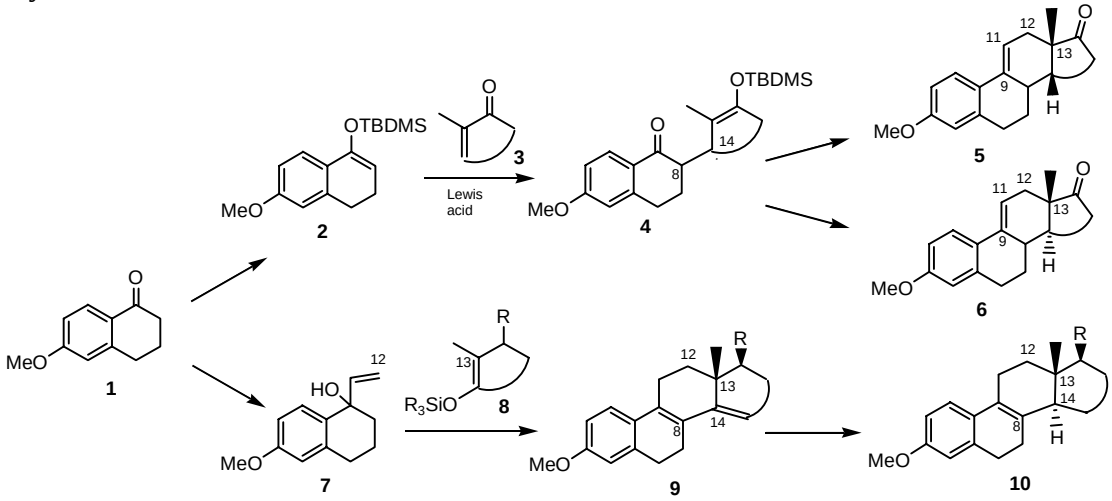
New, efficient procedures have been developed for syntheses of C,D *cis* and C,D *trans* coupled steroid and D-homo steroid skeletons. A Mukaiyama reaction between silyl enol ether **2** and enones **3**, with transfer of the silyl group to the enol of the adduct, gave a second silyl enol ether **4** with formation of the C8-C14 bond as the first one. Starting from this intermediate, ring C of the steroid skeleton has been constructed using the reaction of easily generated carbocations with silyl enol ethers as key transformation.



A second synthesis of C17 substituted C,D *trans* coupled (D-homo) steroid skeletons **10** has been developed using again the addition of a carbocation, generated from the Torgov reagent **7**, to silyl enol ether containing ring D precursors **8**. The adducts have been cyclized by treatment with acid, now under formation of the C8-C14 bond as the last one. A chiral five membered silyl enol ether containing ring D precursor **8** has been synthesized from carvone, and applied as starting compound in the synthesis of a chiral C17 functionalized steroid.

RHODIUM-CATALYZED INTRAMOLECULAR CONJUGATE ADDITION OF VINYLSTANNANES TO 2,3-DIHYDRO-4-PYRIDONES. AN EFFICIENT ROUTE TO STEREOSELECTIVE CONSTRUCTION OF AZABICYCLIC RING SYSTEMS

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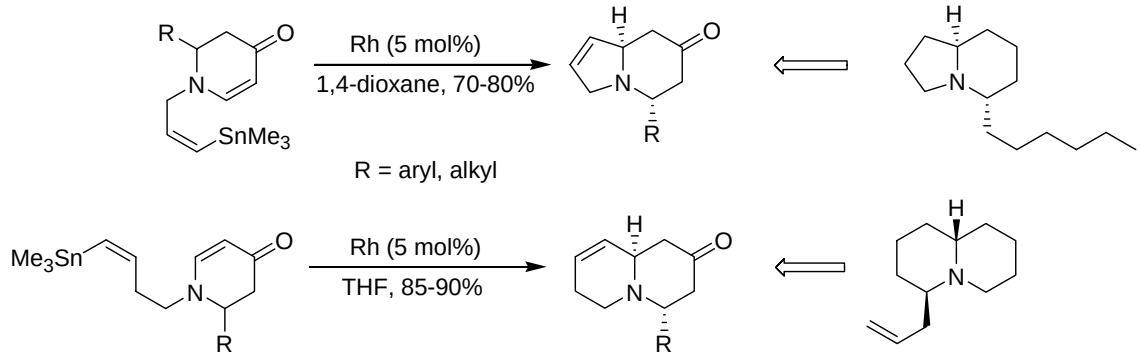
Indolizidines and quinolizidine skeletons can be found in many important natural products. These nitrogen derivatives occur in plants, insects and amphibians and exhibit notable biological activities. Therefore, the stereoselective synthesis of these bicyclic skeletons has become an important goal for synthetic chemists in the recent year.
In connection with our interest in the synthesis of azabicyclic ring systems[1] herein, we report a general and highly stereoselective approach to the construction of indolizidine and quinolizidine rings, based on intramolecular conjugate addition of vinylstannanes to 2,3-dihydro-4-pyridones catalyzed by rhodium(I)-complex [2].



A plausible catalytic cycle and the stereochemical outcome observed in these cyclizations will be reported.

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THE NEW SHOP METHODOLOGY: SELECTING ACTIVE COMPOUNDS FROM A DATABASE FOR DRUG DISCOVERY

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Discovering and bringing one new drug to the public takes an average of 10 to 12 years. Out of every 5000 new compounds identified during the discovery process, only five are considered safe for testing in human volunteers after preclinical evaluations. Therefore any strategy to reduce both the time and the number of compounds would have a great impact in the drug discovery process. Thus, the time reduction in drug discovery might be achieved by screening a huge number of compounds. However, there are more than 250 databases of data, many of which are doubling in size annually [1] and we now have the complete genome sequences of more than 100 organisms [2]. So, computational techniques are really useful to improve this databases analysis.

In this field, the success of quantitative-structure-activity relationships (QSAR) has been linked to the development of appropriate molecular descriptors [3,4]. Nevertheless, most of the 3D descriptors suffer from the drawback of requiring the superimposition of the 3D structures of the ligands according to an hypothesis for their binding mode. It is widely recognized as one of the most difficult and time-consuming steps. Attending to it, new strategies are being developed to succeed designing new active compounds.

In this communication, we will present a new general methodology (SHOP, Scaffold HOPping) useful to select active compounds from a database. Moreover, in order to validate the strategy, two datasets will be analyzed: antiplatelet antagonists that bind to glycoprotein IIb/IIIa [5] and adenosine derivatives with antimalarial activity [6]. After this validation, we also present the application of the new SHOP technique to select the most useful carboxylic acids to be anchored to the androstane skeleton in order to elicit brassinosteroid activity.

Acknowledgements: This work was supported by a grant (IJ) from Generalitat de Catalunya (DURSI, Nº 2002FI 00701) and European Social Fund. Moreover, we want to thank Ministerio de Ciencia y Tecnologia of Spain for supporting this project (BQU2003-07852).

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METHODOLOGY FOR A SOLID-PHASE SYNTHESIS OF "DADDY LONGLEGS" SPIDERS DEFENSE SUBSTANCE

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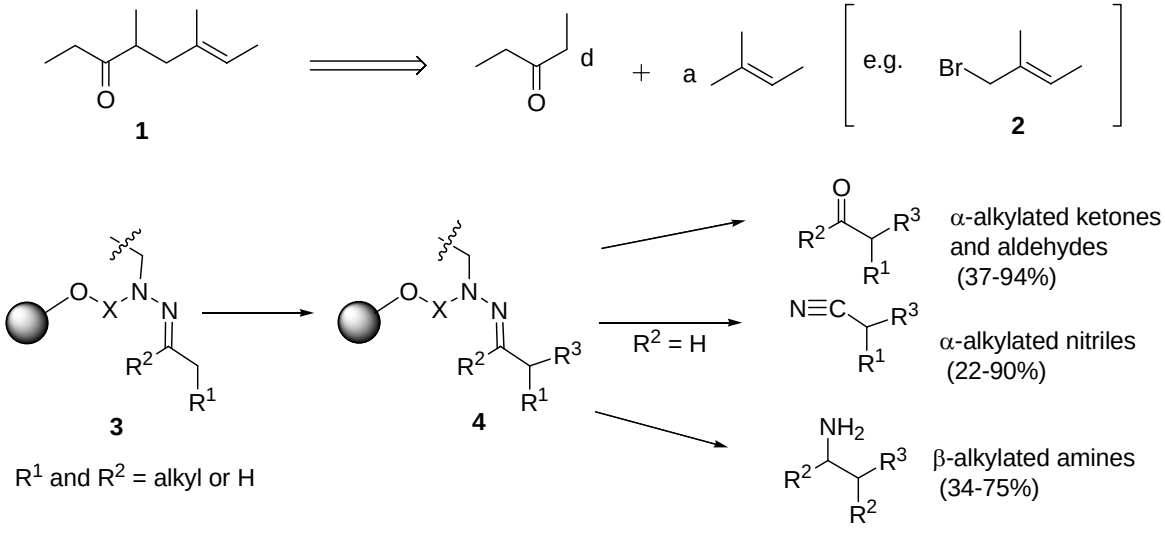
A defense substance of "daddy longlegs" spiders (*Leiobunum vittatum* and *Leiobunum calcar*) (*E*)-4,6-dimethyl-6-octen-3-one (**1**) and its structural analogues can be synthesized by the alkylation of 3-pentanone or other ketones with suitable allylic halides (e.g. **2**) [1]. In order to perform such syntheses on solid support development of a methodology for alkylation of ketones anchored on solid support is necessary. The presented work concerns elaboration of linkers suitable for immobilization and alkylation of ketones and aldehydes on solid support [2]. The carbonyl compounds are anchored via a hydrazone linker (**3**) that modifies reactivity of the substrates and allows for the alkylation with alkyl halides. The alkylated ketones or aldehydes can be cleaved from the polymeric support (**4**) with solution of trifluoroacetic acid in dichloromethane. Modification of the cleavage protocols allows for preparation of different classes of compounds including α-alkylated alcohols, acids, nitriles, and β-alkylated amines [3].



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EFFECTS OF 12-SUBSTITUTED DERIVATIVES OF 5α-PREGNAN-20-ONE ON $GABA_A$ RECEPTOR: SYNTHESIS AND ACTIVITY

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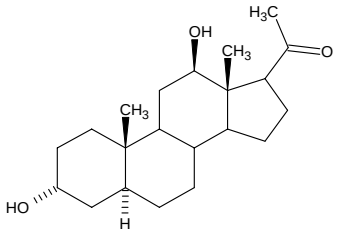
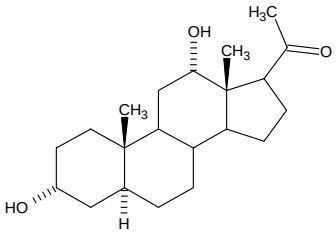
(25R)-3β-Hydroxy-5α-spirostan-12-one (hecogenin) served as starting material for the synthesis of 11- and 12-substituted derivatives of the endogenous neurosteroid - 3α-hydroxy-5α-pregnan-20-one ("allopregnanolone"). Various synthetic methods were investigated in search for the most suitable synthetic approach. Final products (compounds A,B,C and D) were tested for [^{3}H]flunitrazepam binding at the $GABA_A$ receptor in the primary culture of cortical neurons. The activity sought was found in all but compound D.



A



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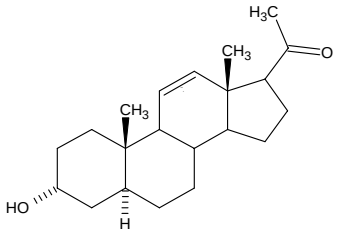

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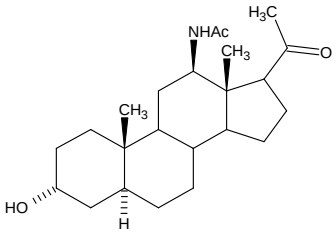

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Acknowledgements:
This work was supported by research project Z4 0550506 and grant GA AS CR IAA4055305.

CATALYTIC ASYMMETRIC ALDOL-TISHCHENKO REACTION CATALYZED BY (AMINOALCOHOL)LANTHANIDE COMPLEXES

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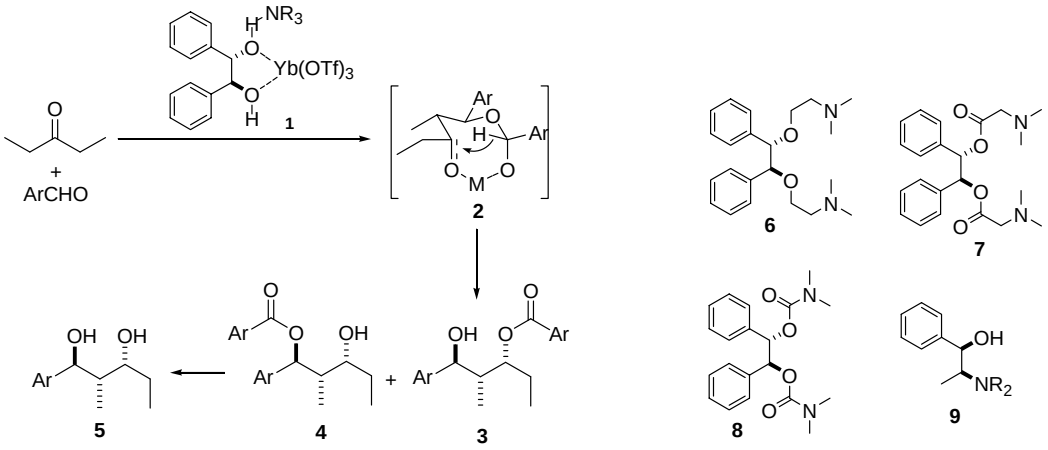
Metal-catalyzed asymmetric *direct* aldol reaction of aldehydes with unmodified ketones still remains a challenge for synthetic chemists. Remarkable success in this area is a result of Trost's and Shibasaki's outstanding work on homo- and heterobimetalic catalyst [1]. Most of the catalytic systems reported to date, however, are limited to rather simple donors such a methyl ketones and some α-substituted methyl ketones. Direct aldol condensation of ethyl ketones still viewed as a formidable synthetic challenge. As a part of our studies on catalytic aldol reactions we recently perceived the aldol-Tishchenko reaction as useful methodology, and we started inquiring studies to design new catalytic systems for enantioselective variant of this process. Our preliminary experiments revealed that *in situ* generated hydrobenzoin/ytterbium(III) complex (**1**) is average efficient catalyst for such process, and 1,3-diols (**5**) are formed with good yield, high diastereocontrol and some level of ee (10-70 %) (Scheme 1).



Scheme 1.

During our research we designed syntheses of novel chiral ligands (*inter alia* **6**-**8**) which enable efficient, catalytic aldol-Tishchenko condensation. A metal/ligand complexes were prepared which possess two sites of opposite character - a basic site and an acidic site, each capable of independent activation in close proximity the ketone and the aldehyde substrate.

The first application of norephedrine-type aminoalcohols (**9**) to enantioselective aldol-Tishchenko reaction between aldehydes and aliphatic ketones leading to condensation products with up to 85 % ee will also be presented.

References:

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SYNTHESIS AND BIOACTIVITY OF ANDROSTANE BRASSINOSTEROID ANALOGS HAVING COMMERCIALLY AVAILABLE ESTER SIDE CHAIN, SELECTED BY MOLECULAR MODELING TECHNIQUES

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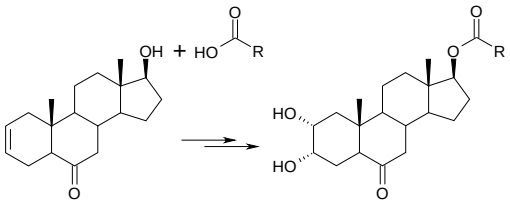
Brassinosteroids (BRs) are considered as plant growth-promoting hormones and brassinolide, isolated several years ago [1], exhibits the most significant activity of the natural ones. Because of their characteristics (increasing the growth yields and making the plant resistant to stressful conditions [2]), its use in the agriculture is nowadays becoming more and more frequent.

In this context, a set of androstane brassinosteroid analogs have been developed and promising results have been obtained [3]. The interest of such compounds is focused on their feasibility to be synthesized in a more economic way as well as on giving more information about the mode of action of brassinosteroids in plants.

These compounds have an ester bond in the side chain moiety and they can be achieved from the steroid alcohol and different carboxylic acids to derive various analogs. Trying to achieve active analogs with a good synthetic cost/activity ratio, we have been working in the design of new androstane compounds having the most convenient functionalized side chain.

In this communication, the synthesis and bioactivity evaluation in the rice lamina inclination test of some androstane analogs will be described. They were achieved by the esterification of properly functionalized skeleton and commercially available acids, which were chosen by means of a new molecular modeling technique, developed considering the structural feature of the side chain for high activity [4]. The activity elicited by some of them assesses the methodology developed, and help us to give more information about the structural requirements in the BRs-receptor interaction.



Acknowledgements: This work was supported by Ministerio de Ciencia y Tecnologia of Spain (BQU2003-07852) and a grant (M.M.) from Generalitat de Catalunya (Nº 2003FI00930) and European Social Fund.

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[4] See Iban Jové *et a.ll* presented in this Conference

SYNTHESIS AND APPLICATIONS OF NEW CYCLOPROPYL-SUBSTITUTED ISONITRILES IN UGI MULTICOMPONENT REACTIONS

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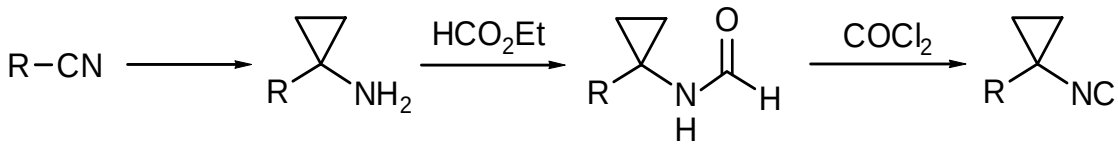
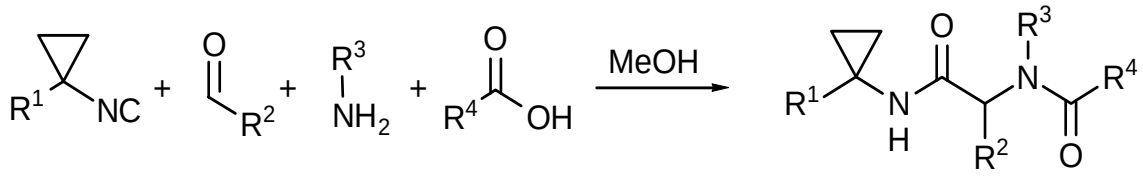
The so-called Ugi reaction is widely used for the synthesis of natural products and especially their analogues [1]. Compounds containing a cyclopropane moiety often exhibit interesting biological activities. Therefore we developed an approach to new cyclopropylisonitriles to be applied in Ugi multicomponent reactions.



The reactivities of these cyclopropylisonitriles were tested in the 4CC Ugi reaction, a number of cyclopropyl-group containing simples dipeptides were synthesized.



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[1] For a review see: A. Dömling, I. Ugi, *Angew. Chem. Int. Ed.* **2000**, 39, 3168–3210

ASYMMETRIC 1,3-DIPOLAR CYCLOADDITIONS OF CHIRAL NITRILE OXIDES DERIVED FROM 8-PHENYLMENTHOL AND (2R)-BORNANE-10,2-SULTAM TO CYCLOALKENES

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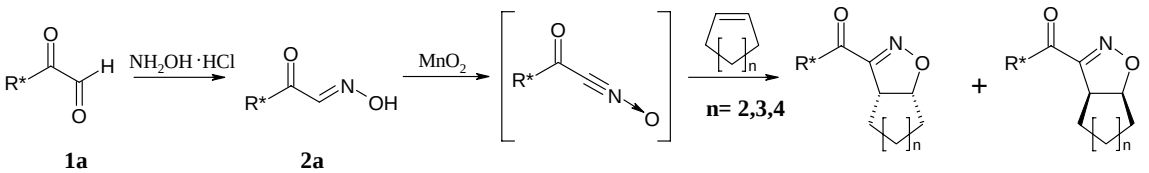
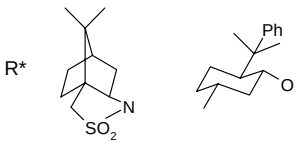
Asymmetric 1,3-dipolar cycloaddition of nitrile oxides to alkenes provides a powerful tool for the stereocontrolled synthesis of 4,5-dihydroisoxazoles [1]. With respect to the easy cleavage of their weak N-O bond, their readily hydrolysis as well as potential nucleophilic or electrophilic reactive centers, these heterocycles demonstrate a high practical usefulness for the syntheses of several types of ligands, pharmaceuticals [2] or natural products [3].

In this communication we would like to report a few representative examples of diastereoselective 1,3-dipolar cycloaddition of optically active nitrile oxides to cycloalkenes.



1a
1b

2a
2b



a **b**

(*R*,*R*) - 6a	**n=2**	**(*S*,*S*) - 6a**
(*R*,*R*) - 6b	**n=2**	**(*S*,*S*) - 6b**
(*R*,*R*) - 7a	**n=3**	**(*S*,*S*) - 7a**
(*R*,*R*) - 7b	**n=3**	**(*S*,*S*) - 7b**
(*R*,*R*) - 8a	**n=4**	**(*S*,*S*) - 8a**
(*R*,*R*) - 8a	**n=4**	**(*S*,*S*) - 8a**

Nitrile oxides were generated from corresponding aldoximes via mild oxidation with MnO_2 and trapped *in situ* with cycloalkenes to furnish 2-isoxazolines. The cycloadducts were obtained in good chemical yields and moderate diastereomeric excesses.

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A FINE-TUNED MOLYBDENUM HEXACARBONYL/PHENOL INITIATOR FOR ALKYNE METATHESIS

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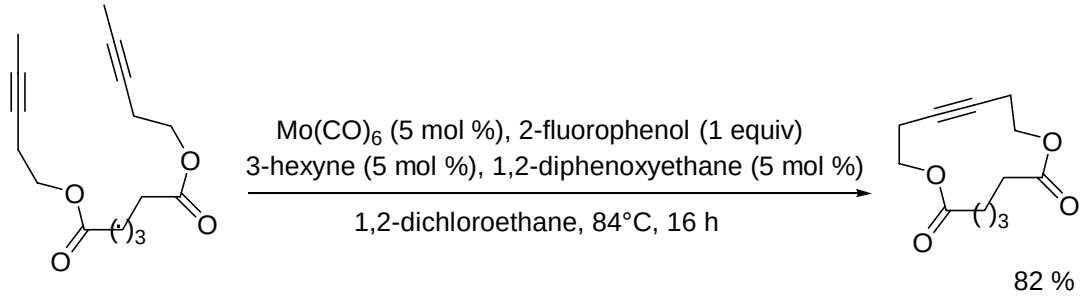
A*lkyne metathesis*, as compared with the sister transformation–*alkene metathesis*– is new and still less-investigated reactions in organic chemistry, thus some important applications in the synthesis of organic and organometallic compounds and in materials science have already been found, for example in target oriented syntheses [1]. The most active catalysts used in this transformation are extremely air- and moisture sensitive and commercially unavailable tungsten or molybdenum complexes [1]. A structurally unknown catalyst from molybdenum hexacarbonyl and phenol, first proposed by Mortreux [2], is very attractive because it can be formed in situ from stable and cheap off-the-shelf constituents and used in commercially grade solvents.

Therefore, we were prompted to refine this "user-friendly" catalyst further in order to extend its scope and to enhance reactivity toward more elaborate and sensitive substrates. In our preliminary investigations the $Mo(CO)_6$/2-fluorophenol system was seemed to be very promising [3, 4].

The use of 2-fluorophenol can be further combined with other activation methods to allow alkyne metathesis at relatively low temperature (84°C) [5]. Application of this *"fine-tuned" catalyst* shows high activity in several alkyne cross-metathesis (ACM), homo-metathesis (HM) and ring-closing (RACM) reactions [5]. Compatibility of C-silylated and terminal alkynes with our catalytic system was also studied.



References:

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[4] For recent applications of our optimized system, see: [4a] Brizius, G.; Billingsley, K.; Smith, M. D.; Bunz U. H. F. *Org. Lett*. **2003**, 5, 3951-3954, [4b] Bly, R. K.; Dyke, K. M.; Bunz U. H. F. *J. Organomet. Chem*. **2005**, 690, 825-829.

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SYNTHESIS AND HYDROPHILIC DERIVATIZATION OF 3α,7α-DIHYDROXY-5β-PREGNAN-20-ONE

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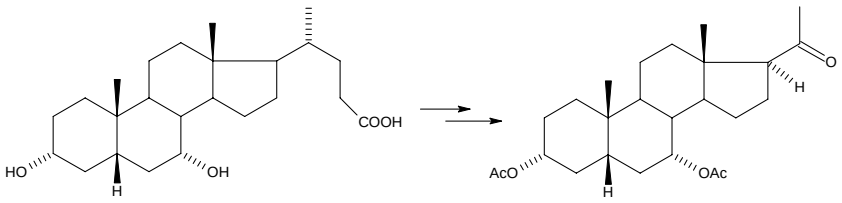
The activity of NMDA-receptor is significantly influenced [1] by the derivatives of 5β-pregnan-20-one, especially those that are substituted by a polar group in position 3 (e.g. a sulfonyloxy group [2]). Therefore there were prepared some new derivatives of 3α,7α-dihydroxy-5β-pregnan-20-one which should exert biological activity on NMDA-receptor [1,2].

The starting chenodeoxycholic acid **I** was transformed according to literature [3]: a carboxylic group was converted to a methoxycarbonyl group which on reaction with Grignard reagent produced the corresponding diphenyl derivative. Following allylic bromination, dehydrohalogenation and oxidation lead to 3α,7α-dihydroxy-5β-pregnan-20-one diacetate (**II**).



I **II**

The compound **II** was subsequently converted to derivatives with hydrophilic (sulphate, hemisuccinate) substituents in positions 3 and 7.

Acknowledgements:
This work was supported by research project Z4 0550506 and grant GA AS CR IAA4055305.

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STILLE-HECK CROSS-COUPLING SEQUENCES: A VERSATILE NEW APPROACH TO STEROIDS AND STEROID ANALOGUES

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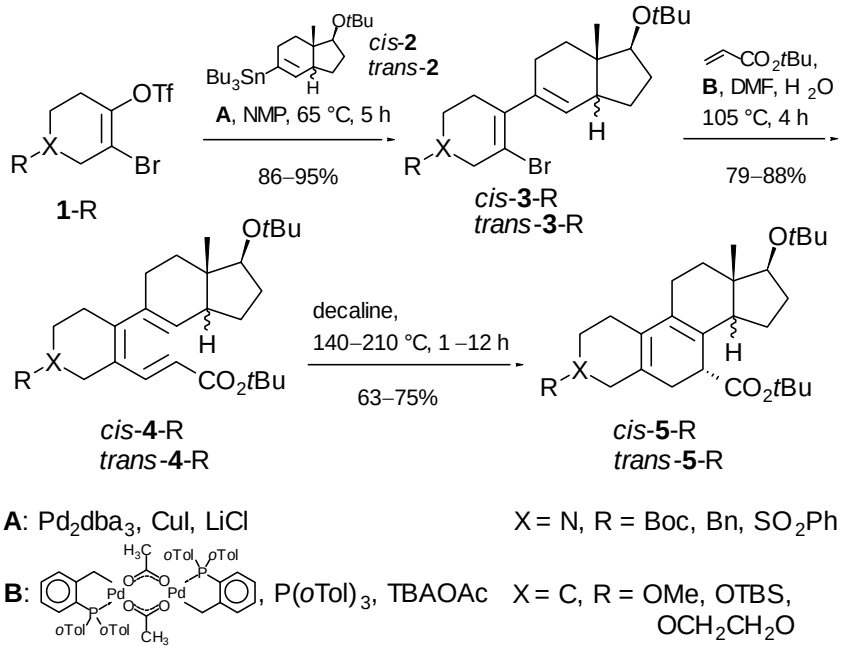
Most of the previously reported synthetic routes to steroids lead to specific target molecules only. Modern lead structure optimization, however, requires access to a broad diversity of structural modifications of any given class of compounds. With this in mind, we embarked on a project to develop a highly flexible building block approach to a number of structurally diverse steroid analogues.



The chemoselective STILLE-HECK cross-coupling sequences of 1-bromo-2-trifluormethanesulfonyloxycyclohexenes (**1**-R) with the bicyclic alkenylstannanes (*cis*- or *trans*-***2***-*R*) and acrylates lead to tricyclic 1,3,5-hexatrienes (*cis*- or *trans*-**4**-*R*) which cleanly undergo 6π-electrocyclizations at elevated temperatures. Interestingly, the products (*cis*- or *trans*-**5**-*R*) were obtained as single diastereomers [1]. Since a variety of appropiate starting materials is easily available, new substituted analogues of testosterone, estradiol as well as new steroidal aminoacid derivatives have been prepared.

References:

[1] (a) H. W. Sünnemann, A. de Meijere, *Angew. Chem.* **2004**, *116*, 913–915; *Angew. Chem Int. Ed.* **2004**, *43*, 895–897; (b) A. de Meijere, M. Schelper, M. Knoke, B. Yucel, H. W. Sünemann, R. P. Scheurich, L. Arve, *J. Organomet.Chem.* **2003**, *687*, 240–255.

SYNTHESIS OF ANALOGUES OF A POTENT ANTITUMOR SAPONIN OSW-1

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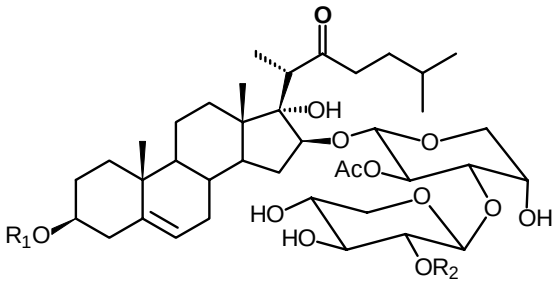
Saponin OSW-1 belongs to family of glycosides isolated 12 years ago by Sashida *et. al*. from the bulbs of *Ornithogalum Saundersiae*. The saponins appeared to be strongly cytotoxic against broad spectrum of malignant tumor cells [1].



R_1	R_2
H	4-methoxybenzoyl **(OSW-1)**
H	3,4-dimethoxybenzoyl
H	(E)-cinnamoyl
Glc	4-methoxybenzoyl
Glc	(E)-cinnamoyl

So far several methods of synthesis of saponin OSW-1 and its analogues have been elaborated [2]. Our group has accomplished synthesis of this natural product and than has undertaken further efforts towards synthesis of various analogues for biological studies [3]. OSW-1 analogues with different size of side chain, structural isomers and 22-oxa analogues as well as the derivatives with modified sugar part were obtained and biologically tested. Unfortunately, all synthesized compounds were found to be less cytotoxic than the naturally occurring saponin.

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SYNTHESES OF PREGNANOLONE AND EPIPREGNANOLONE HAPTENS

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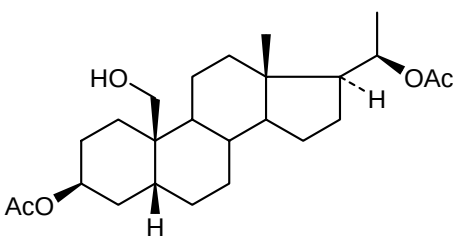
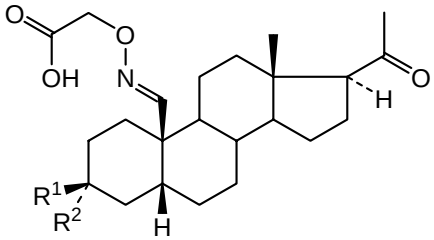
Syntheses of title haptens represented by corresponding 19-(O-carboxymethyl)-oximes (19-CMO) were developed. The haptens will serve as a base for the completing of RIA kits, which will enable routine monitoring of parent steroids in body fluids and consequently broadening of our knowledge about these neuroactive steroids.

The starting compound for the syntheses was (20*R*)-5β-pregnane-3β,19,20-triol 3,20-diacetate (**1**), available from commercial pregnenolone acetate in six steps [1,2]. For the epipregnanolone hapten, the 19-CMO moiety was introduced first, protected in form of methyl ester, and acetates were removed selectively in an acidic medium. The diol was reacted with *tert*-butyldimethylsilyl chloride, the 3-monosilylated compound was separated, oxidized in position 20, and protecting groups were successively removed, giving second of the title haptens, (19*E*)-3β-hydroxy-20-oxo-5β-pregnan-19-al 19-[O-(carboxymethyl)oxime] (**2**).

For the epimeric pregnanolone hapten, 19-hydroxy group in starting compound **1** was protected as *tert*-butyldimethylsilyl derivative, after alkaline hydrolysis the 3-hydroxy derivative was separated, oxidized, and resulting 3-ketone was reduced with sodium borohydride to the corresponding 3α-hydroxy derivative. Protection as methoxymethyl derivative, liberating of 19-hydroxyl, and transformation into 19-CMO derivative followed. Deacetylation, esterification of CMO moiety with diazomethane, and oxidation in position 20 were the next steps. Final successive deprotections gave pregnanolone hapten, (19*E*)-3α-hydroxy-20-oxo-5β-pregnan-19-al. 19-[O-(carboxy-methyl)oxime] (**3**).



1



2, R^1 = OH, R^2 = H;
3, R^1 = H, R^2 = OH

Acknowledgements: The project was supported by the Grant Agency of the Czech Republic, grant No. 203/04/0123.

References:

[1] Terasawa T., Okada T.: Tetrahedron 1986, 42, 537.
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NEUROACTIVE STEROIDS: 7-AZA-ALLOPREGNANOLONE – A WEAK SUBSTITUTE FOR ALLOPREGNANOLONE

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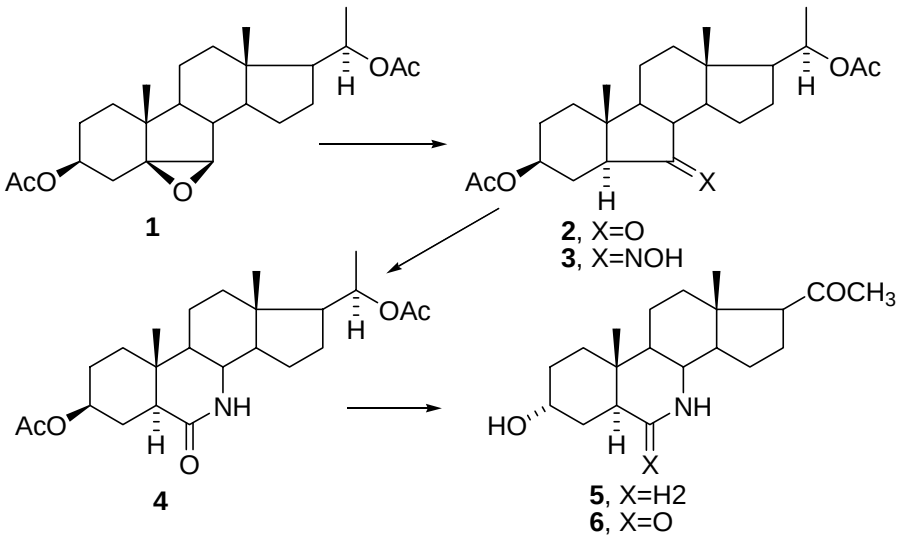
Steroid chemistry – once the golden egg of pharmaceutical sciences – was several times considered outworn and still it enjoyed its renaissance repeatedly. The structures of steroid hormones were emulated by organic chemists many times, and various types of modification were produced: new types of substitution of the classical steroid skeleton were examined or the skeleton itself was modified by increasing or reducing the flexibility of the molecule or even reversing its chirality. Various changes were made in the steroid side chain. Nature herself showed many times that a nitrogen atom in proper position often secures strong biological effects: the nitrogen atom was part of a substituent as well as or part of the steroid skeleton. Here we present the synthesis of 7-aza-allopregnanolone (**5**):

7-Nor-20-oxopregn-5-en-3β-yl acetate was converted into (*20R*)-5β,6β-epoxy-5β-7-norpregnane-3β,20-diyl diacetate (**1**) in three steps. Stereospecific migration of the 6α-hydride ion led to a 6-oxo derivative with 5α-configuration (**2**). (*Z*)-oxime of this ketone (**3**) underwent the Beckmann rearrangement to yield lactam **4** with nitrogen in position 7. Lithium aluminium hydride reduction, followed by oxidation and regioselective reduction of 3,20-diketone produced 7-aza-3α-hydroxy-5α-pregnan-20-one (**5**) which inhibited binding of [^{35}S]TBPS to the $GABA_A$ receptor. The corresponding lactam - 7-aza-3α-hydroxy-5α-pregnane-6,20-dione (**6**) – was inactive.



Acknowledgements:
This work was supported by grants No. **IAA 4055305** and Z4 055 905.

NEUROACTIVE STEROIDS: 16α-SUBSTITUTED ANALOGUES OF ALLOPREGNANOLONE

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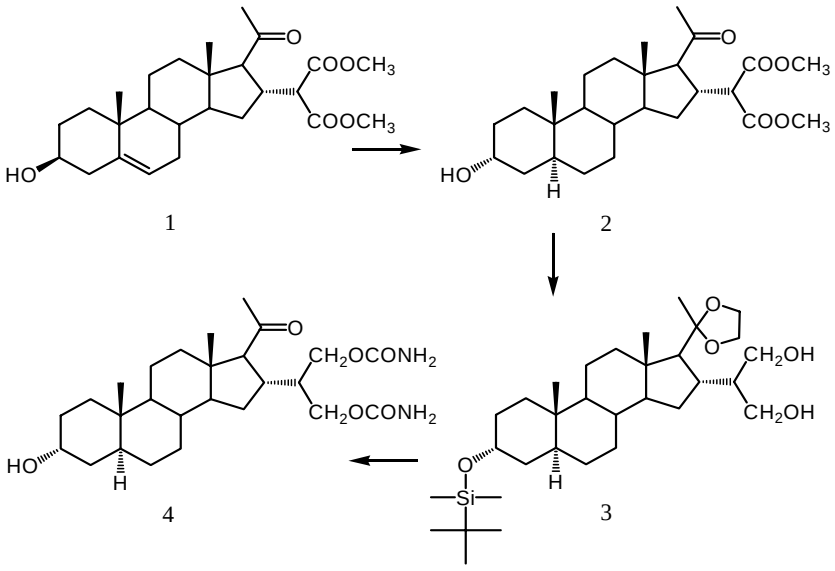
Endogenous anesthetic neurosteroids (e.g. allopregnanolone, 3α-hydroxy-5α-pregnan-20-one) function by positive allosteric modulation of γ-aminobutyric acid (GABA) via its receptors in neuronal membranes. Thus, these compounds help to stop the transmission of excessive signals of pain and anxiety. Practical use of these compounds has been hampered by their very fast metabolism and their low solubility in body fluids.

According to our previous experiments, convenient substitution of position 16α does not reduce the binding of such products to $GABA_A$ receptors and can improve their solubility. The aim of this work was to synthesize compound of this type.

A starting compound, 3ß-hydroxy-16α-bis(methoxycarbonyl)methyl-pregn-5-en-20-one (**1)** was hydrogenated using a palladium catalyst. Then inversion of configuration at carbon C-3 was carried out by the Mitsunobu reaction (**2**). After introduction of protecting groups in positions 3 and 20, the lithium aluminum hydride reduction of the carboxylic groups produced a dihydroxy derivative **3**. The treatment of this diol with $COCl_2$ in toluene and in dimethylanilin afforded a carbamoyl derivative, which after deprotection of groups in positions 3 and 20 gave desired the 3α-hydroxy-16α-bis(carbamoyloxymethyl)methyl-5α-pregnan-20-one (**4**).



Acknowledgements:

This work was supported by grants No. **IAA 4055305** and Z4 0550506.

STEROID SULPHATES HYDROGENATION

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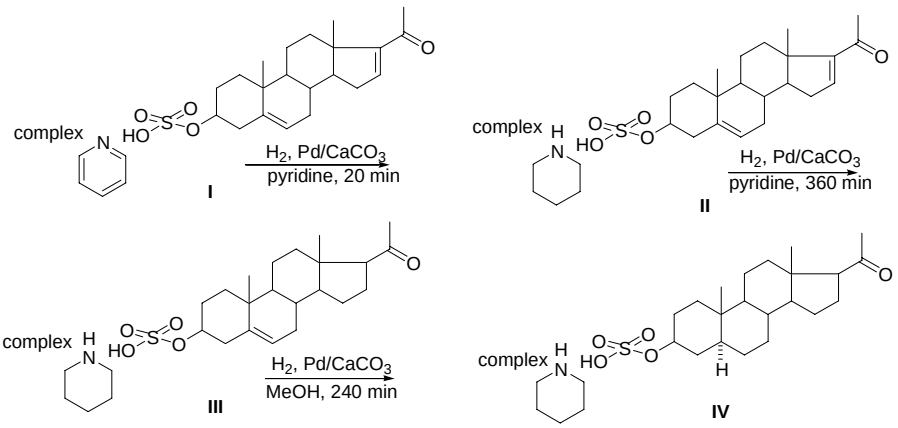
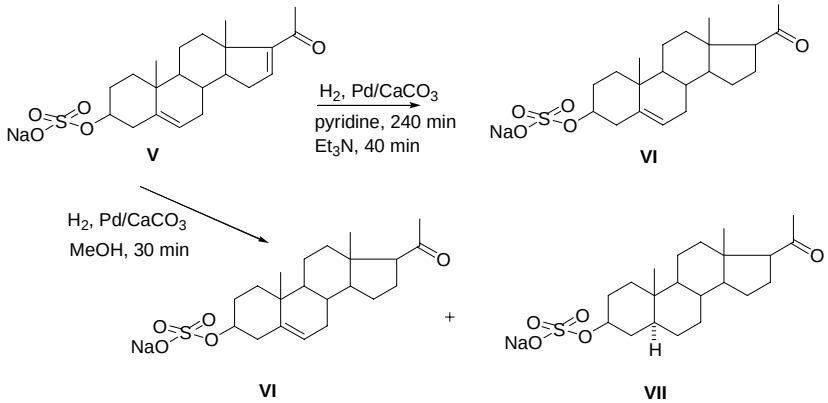
Native steroid ligands of neuronal receptors exert very important activity. Research of the ligands and receptors demands labelled derivatives. Tritium is frequently used as the label. We now report on selective methods for the introduction of tritium to the pregnenolone sulphate (3β-hydroxy-pregn-5-en-20-one sulphate). Model experiments were done with hydrogen.

Pyridinium dehydropregnenolone sulphate (**I**) was at first hydrogenated in pyridine on the pyridinium ring (see compound **II**), this reaction was followed by selective hydrogenation on the 16(17) double bond in compound **III**. Hydrogenation of the 5(6) double bond in compound **IV**, was achieved only when solvent pyridine was replaced with methanol.



Sodium salt of dehydropregnenolone sulphate (**V**) was better substrate, as it contains only double bonds in the steroid part of molecule. This derivative was selectively hydrogenated (**VI**) in pyridine and triethylamine. In spite of short reaction time hydrogenation in methanol gave the mixture of di- and tetrahydroderivatives **VI** and **VII**, respectively.



Acknowledgements:
The work was supported by research project Z4 0550506 and grant GA AS CR IAA4055305

SYNTHESIS OF THE 3-CARBOXY DERIVATIVES OF 5β-PREGNAN-20-ONE

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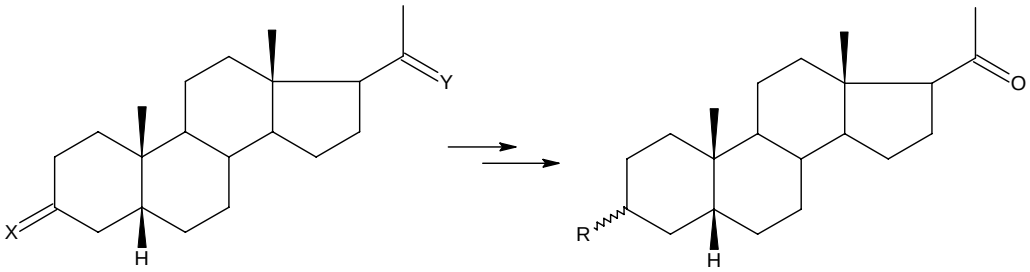
Within the synthesis of steroids active at NMDA receptor [1,2], we prepared 3α- and 3β-carboxy derivatives **V** and **VI** in the following way:

The starting 5β-pregnan-3,20-dione (**I**) was protected at position 3 and subsequently the 20-oxo group was reduced by a one-pot reaction to a 20-hydroxy derivative **II**. Ketone **III**, obtained by deprotection of 3-oxo group and benzoylation of the 20-hydroxy group, was transformed to cyanohydrin **IV**. It was then dehydrated and hydrolyzed to the carboxylic acid that was converted to the methyl ester. Hydrogenation and oxidation afforded two epimers **V** and **VI**. The 3α-carboxy epimer (**V**) prevailed in the reaction mixture in the ratio 3 : 1.

The structure and configuration of both carboxy derivatives were confirmed by the NMR-spectroscopy.



I, X = O, Y = O
II, X = $(OMe)_2$, Y = H, OH
III, X = O, Y = H, OBz
IV, X = CN, OH, Y = H, OBz

V, R = α-COOMe
VI, R = β-COOMe

Acknowledgements:
This work was supported by research project Z4 0550506 and grant GA AS CR IAA4055305.

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[1] Irwin R.P., Lin S., Rogawski M.A., Purdy R.H., Paul S.M.: *J. Pharmacol. Exp. Ther. 271*, 677 (1994).
[2] Weaver C.E., Land M.B., Purdy R.H., Richards K.g., Gibbs T.T., Farb D.H: *J. Pharmacol. Exp. Ther. 293*, 747 (1998).

THE PREPARATION OF THE SPIROSTANIC ANALOGUES OF BRASSINOLIDE AND CASTASTERONE

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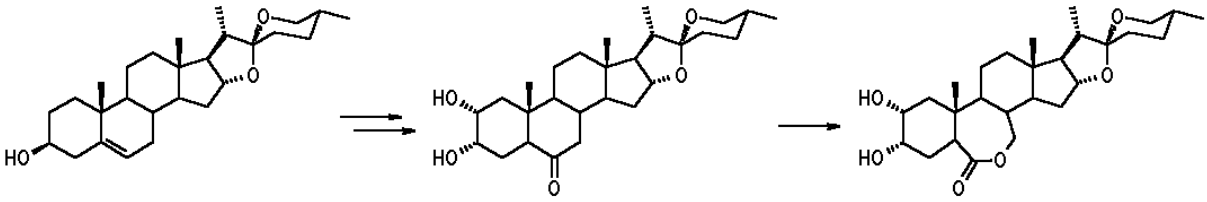
Methods for the preparation of two of the most widely used spirostanic analogues of brassinosteroids [1,2], namely (25*R*)-5α-spirostan-6-one-2α,3α-diol (**2**) and (25*R*)-B-homo-5α-spirostan-6-oxo-7-oxalactone-2α,3α-diol (**3**), starting from diosgenin, were examined. The best preparative route was via diosgenin (**1**) tosylation, isosteroidal rearrangement with potassium acetate in aqueous acetone, oxidation with Jones reagent, cyclopropyl ring opening with hydrobromic acid, hydrogen bromide elimination with lithium bromide and carbonate, dihydroxylation with osmium tetroxide and N-methylmorpholine N-oxide, producing (25*R*)-5α-spirostan-6-one-2α,3α-diol in 57.3% overall yield and lactonization with trifluoroperoxyacetic acid producing (25*R*)-B-homo-5α-spirostan-6-oxo-7-oxalactone-2α,3α-diol in 24.6% overall yield from diosgenin. The shortest route to (25*R*)-5α-spirostan-6-one-2α,3α-diol results in only 39.4% overall yield.



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Acknowledgements: Support from FAPESP is gratefully acknowledged.

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A NEW TYPE OF STEROIDS WITH A CYCLOBUTANE FRAGMENT IN THE AB-RING MOIETY

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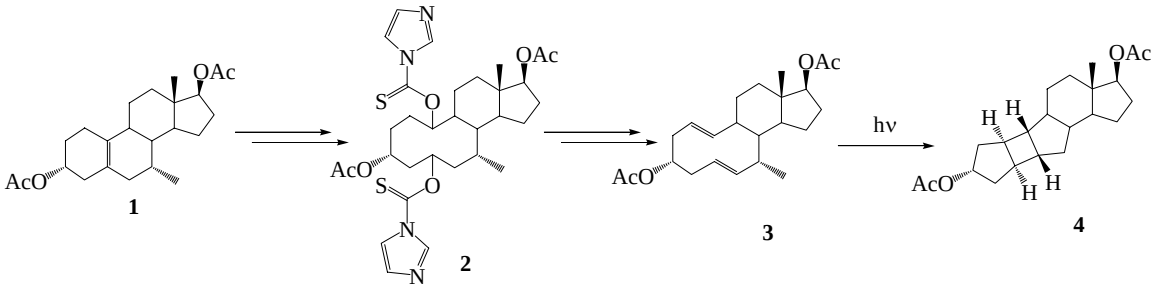
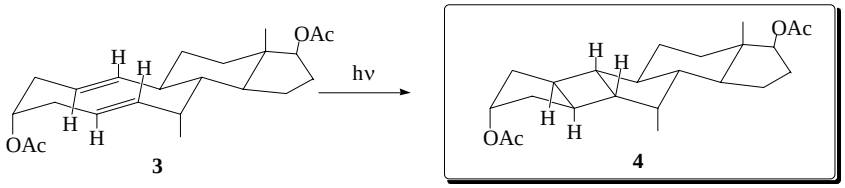
As a part of our ongoing research on new 5,10-seco steroids, we became interested in derivatives with two double bonds in the ten membered macrocycle. Inspection of molecular models of such compounds showed common conformational features with normal steroids. We now describe the synthesis of 5,10-seco steroid containing $\Delta^{1(10)}$- and $\Delta^{5(6)}$-double bonds in the AB ring, and its subsequent photochemical transformation into a new non-olefinic product containing a cyclobutane fragment in the cyclic part of the molecule.



The double unsaturated ten membered ring will preferentially adopt “an elongated chair conformation” which is close to that for steroids. The proximity of the two parallel lying double bonds allows a successful [2+2] photochemical cycloaddition to give a cyclobutane fragment in the cyclic part of the molecule.



Although compound **4** looks quite different from normal steroids, its pentacyclic skeleton shows much conformational similarity with that of compounds of this series.

ALLYL DERIVATIVES OF CHOLIC ACID AS BUILDING BLOCKS FOR THE CONSTRUCTION OF CHOLAPHANES

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Bile acids, readily available natural products, are useful starting materials for the synthesis of functionalized macrocyclic host molecules [1]. These so-called "cholaphanes" are interesting compounds [2] because of their chemical features, such as: structural rigidity, amphiphilicity, chirality, and orientation of their hydroxy groups towards the center of a concave face. These supramolecules have application in the synthesis of molecular receptors, enzyme models, or transporters [3,4].

Allyl derivatives (3α and/or 24) of 5β-cholan-3α,7α,12α,24-tetraol were used in the synthesis of four cyclic supramolecular hosts (*Fig. 1*) formed by Ring-Closing Metathesis, as a key step. The two steroidal units were combined *via* their 3α- and 24-hydroxy groups ("head to tail" method) and between themselves ("head to head" and "tail to tail" methods) using *o*-phtalic and/or allylic spacers.

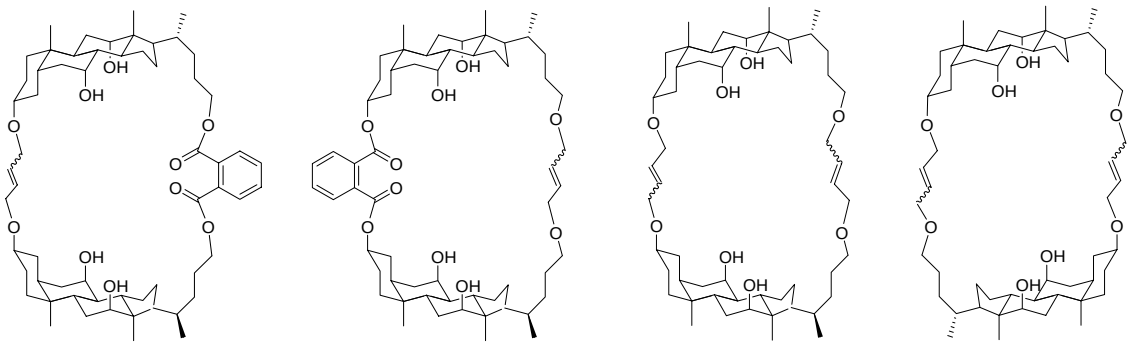

Fig. 1

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THE ABNORMAL BAEYER-VILLIGER REARRANGEMENT OF 23-OXOSAPOGENINS

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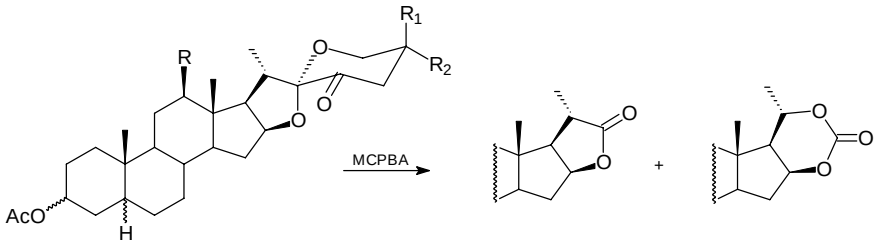
Spirostane sapogenins are widely distributed in plants [1]. For a long time, sapogenins have been used as relatively cheap raw materials for the synthesis of medicinally important steroids. A crucial step in sapogenin transformations leading to steroid drugs is cleavage of the spiroketal system.

Spirostanes can be selectively converted into their 23-oxo derivatives using the Barton's procedure improved later by Iglesias-Arteaga *et al* [2].

The reaction of (25*R*)- and (25*S*)-23-oxosapogenins with MCPBA was studied [3]. The Baeyer-Villiger rearrangement was followed by unusual cleavage of the spiroketal moiety under the reaction conditions. Two major products were obtained: bisnorcholanic lactone and 16,20-diol carbonate. GC-MS analysis of the reaction mixture proved also formation of a low molecular product – 3-methylbutyrolactone.



3α,5β, R = H, R_1 = H, R_2 = Me (25*R*)
3β,5β, R = H, R_1 = Me, R_2 = H (25*S*)
3β,5α, R = AcO, R_1 = H, R_2 = Me (25*R*)

The reactions of the (*25R*)-23-oxosapogenins were considerably faster. The reactions are subject to acid catalysis, which indicates that the rate determining step is the peroxyacid attack. Reaction barriers calculated using the semiempirical PM3 method justify the observed differences in reaction rates. The regioselectivity of the reaction can be explained in terms of the conformational preference of the Criegee's intermediate.

The degradation products may be of interest for the pharmaceutical industry.

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SYNTHESIS OF 23-OXA-22-DEOXO ANALOGUES OF SAPONIN OSW-1

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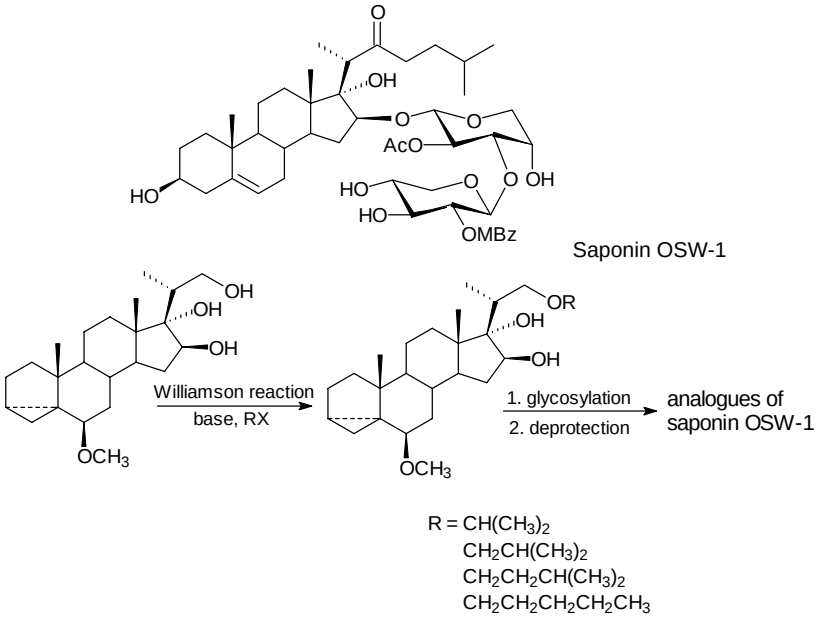
The steroidal saponin OSW-1 belongs to a family of similar compounds isolated from the bulbs of *Ornithogalum saundersiae*. In vitro assays have shown that this saponin is extremely toxic against a broad spectrum of tumor cells [1]. The successful crusade for the synthesis of the natural product, opened the way for chemical synthesis of large number of analogues for biological studies [2,3]. Both steroid aglycone and sugar unit are important for biological activity of OSW-1. In this work synthesis of analogues of saponin OSW-1 will be presented. The sugar part was not changed, but aglycone was suitably modified to synthesize analogues without C-22 carbonyl group, and with the oxygen atom in the position 23.



Previously, we have reported a synthesis of 16β,17α,22-triol, valuable intermediate for the synthesis of the saponin OSW-1 analogues [4]. This compound was regioselectively converted to the ether analogues by Williamson's reaction with alkyl halides under the basic conditions (NaH or *t*-BuOK). The reaction promoted by NaH unexpectedly afforded a cyclic hemiacetal in addition to the ether product. The obtained aglycones will be subjected to glycosylation with the OSW-1 disaccharide under standard conditions [5].

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APPLICATION OF CM AND RCM TO THE SYNTHESIS OF 19-FUNCTIONALIZED DERIVATIVES OF 1α-HYDROXYVITAMIN D

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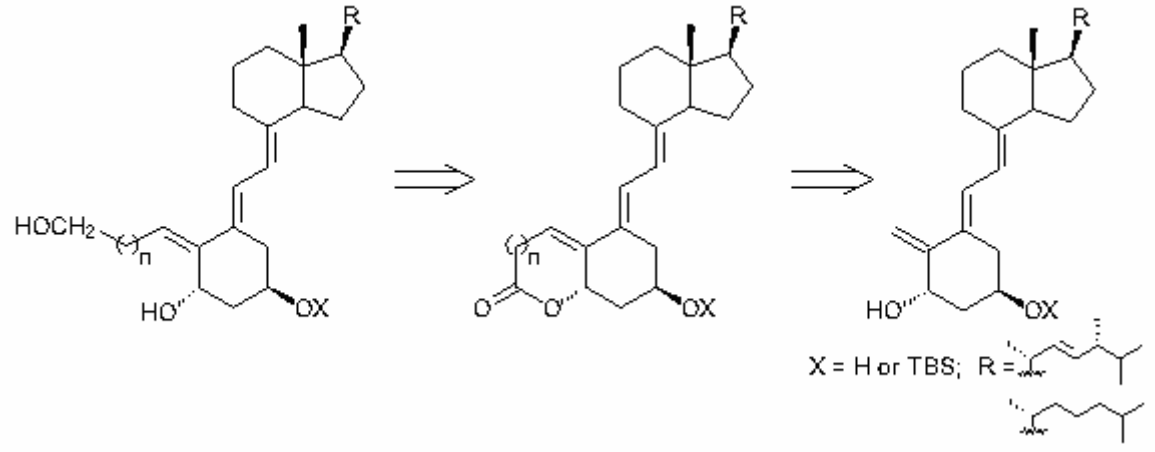
The medicinal importance of various vitamin D derivatives (the drugs based on vitamin D are applied for treatment of cancer, psoriasis, immune dysfunction, endocrine disorders, *etc*) stimulated continuous interest in the synthesis of new analogues.

The synthesis of 19-functionalized derivatives based on CM seems to be the shortest and straightforward way. Unfortunately, this direct approach failed. Therefore a new synthetic strategy for preparation of 19-functionalized derivatives of 1α-hydroxyvitamin D was elaborated. In the methodology developed, synthesis of cyclic analogues proceeds through RCM of 1α-alkenyl derivatives of *trans*-vitamin D, the further reduction of obtained compounds afforded 19-functionalized analogues. The last step is photochemical transformation of *trans*-vitamin D to the desired *cis* isomer.



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COMPUTATIONAL ANALYSIS OF ACTIVE SITES IN VITAMIN D RECEPTOR BOUND WITH ANALOGS OF VARIOUS CALCEMIC ACTIVITIES

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The vitamin D receptor (VDR), a member of nuclear receptor (NR) superfamily, is responsible for calcium homeostasis. Each year more evidences appear that coactivators associated with the VDR significantly influence calcemic activity of vitamins D [1,2]. Recently, several complexes of human and rat VDR have been successfully crystallized in the absence [3] or presence [4] of peptide (KNHPMLMNLLKDN) mimicking the coactivator sequence.

In this work we present analysis of VDR active sites in crystals holding as the ligands: the natural vitamin D hormone, 1α,25-$(OH)_2D_3$, the most potent analog 2MD, (20*S*)-2-CH_2-19-nor-1α,25-$(OH)_2D_3$, and non active analog 2MbisP, (20*S*)-2-CH_2-1α-OH-19-nor-bishomopregnacalciferol. The program BIODESIGNER permitted us to find the nearest (3.5 Å) neighbors of the peptide and vitamin D. To compare detailed conformation of amino acids forming active sites in VDR complexes, we subjected the crystal structures to sequence alignment, then superimposed them and calculated deviations on individual amino acids as well as the RMSD on all atoms. We established that the peptide changes side chain conformation of ca. 20% of VDR amino acids. We also found that in the inactive ternary complex 2MbisP-VDR-peptide, asparagine 420, located on the last helix H12, disturbs a salt bridge contact, known to be the main stabilizing factor of the receptor structure in hormone-VDR dimers.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF *DES*-C,D-ANALOGS OF 2-METHYLENE-19-NOR-1α,25-$(OH)_2D_3$

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The discovery of the hormonal form of vitamin D, 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), has resulted in a major expansion of our understanding of the functions of vitamin D. Besides its activities in regulating calcium and phosphorus metabolism, it is now evident that 1,25-$(OH)_2D_3$ functions in cellular differentiation, in an immuno-modulating manner, and in suppression of cellular proliferation. To develop compounds specific for these disease functions, a number of analogs of 1,25-$(OH)_2D_3$ has been prepared. A broad range of the vitamin D activities has stimulated the structure-activity studies. Considerable attention has been placed on the synthesis of analogs of the natural vitamin D hormone possessing enhanced calcemic potency or exerting selective activity profile.

In our search for vitamin D compounds of potential therapeutic value, in 1998 we synthesized analogs of the natural hormone in which the exocyclic methylene unit is transposed from C-10 to C-2 [1]. It was found that 1α,25-dihydroxy-2-methylene-19-norvitamin D analogs are characterized by significant biological potency, enhanced dramatically in compounds with an "unnatural" (20*S*)-configuration. Recent studies of the analog, **2MD**, showed its ability to induce bone formation both *in vitro* and *in vivo*.

An interesting modification of the vitamin D skeleton is elimination of the C and/or D rings. The first compound, retiferol, lacking the C,D-substructure, was obtained ten years ago [2]. In the following years, several *des*-C,D vitamin D_3 derivatives, including 19-nor analogs, were synthesized [3,4] and some of them (**Ro 65-2299**) showed improved biological activities [5].

As a continuation of our studies on 2-methylene-19-norvitamin D compounds, analogs characterized by the lack of the C,D-ring have been synthesized and biologically tested.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2-(3'-HYDROXYPROPYLIDENE)-19-NOR ANALOGS OF 1α,25-$(OH)_2D_3$

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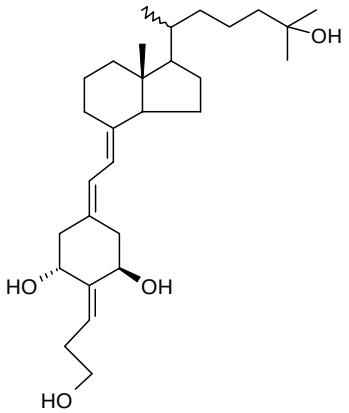
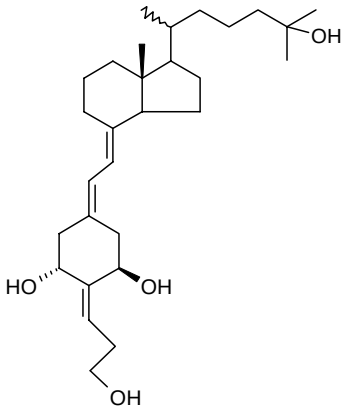
In a search for novel, biologically active vitamin D compounds of potential therapeutic utility, *E*- and *Z*-isomers of 1α,25-dihydroxy-2-(3'-hydroxypropylidene)-19-norvitamin D_3, and the derivative of the former compound, possessing 3'-(methoxymethoxy)propylidene substituent at C-2, were synthesized. All of the vitamins were obtained in two convergent syntheses, both starting from (-)-quinic acid and the protected 25-hydroxy Grundmann ketone. The first route involved Wittig-Horner coupling of the A-ring phosphine oxide, derived from quinic acid, with the corresponding hydrindanone fragment. Since such synthetic path provided almost exclusively *E*-geometrical isomers of 19-norvitamin D compounds, an alternative route was devised based on Julia olefination of the A-ring cyclohexanone derivatives with the corresponding allylic sulphones obtained from Grundmann ketones. This method resulted in formation of both *E*- and *Z*-isomers, that allowed us to perform biological evaluation of all analogs differing in configuration at C-20 and possessing differently oriented A-ring hydroxyalkylidene substituent. All tested compounds bind the vitamin D receptor (VDR) and cause HL-60 differentiation as effectively as the natural vitamin D hormone. Analogs in the *E*-series (**1AGR** and **1AGS**) have significantly higher transcriptional potencies (10 and 50 times) than 1α,25-dihydroxyvitamin D_3. The *E*-isomers, **1AGR** and **1AGS**, are more active in raising serum calcium and intestinal calcium absorption than is the native hormone. The *Z*-isomers, **2AGR** and **2AGS**, have little or no activity *in vivo* and are much less active *in vitro* than the *E* series.



1AGR: 20*R*
1AGS: 20*S*



2AGR: 20*R*
2AGS: 20*S*

A NEW SYNTHESIS OF VITAMIN D C/D RINGS - SIDE CHAIN BUILDING BLOCKS FROM THE HAJOS-PARRISH KETONE. CONSTRUCTION OF *trans*-HYDRINDANE SYSTEM VIA FRAGMENTATION OF ALLYL SULFINIC ACIDS

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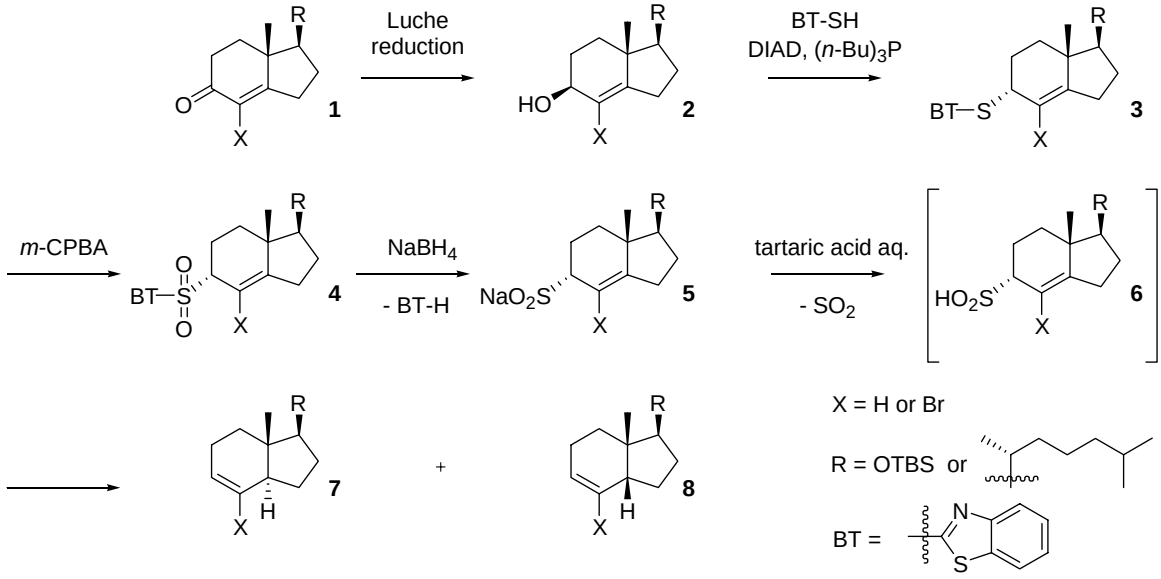
Construction of *trans*-hydrindane vitamin D building blocks from the Hajos – Parrish ketone and congeners has received a great deal of attention [1]. We present now a new versatile method for reductive transposition of the double bond in α,β-unsaturated ketones and α-bromo-α,β-unsaturated ketones, using of allyl sulfinic acids fragmentation [2] as the key step. A hydroxy group in allylic alcohols **2** prepared from **1** by the Luche reduction, was substituted with 2-mercaptobenzothiazole under the Mitsunobu reaction condition and then thiols **3** were oxidized into sulfones **4**. Reduction of the sulfones with sodium borohydride in ethanol [3] gave the respective sodium sulfinates **5**. The latter products (without isolation) upon acidification with aq. tartaric acid gave mainly or exclusively *trans*-hydrindane derivatives **7**.



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NEW SYNTHESIS OF SPIROKETALS VIA ALKYNYLTRIFLOUROBORATES: A COVENIENT ACCESS TO THERMODYNAMICALLY NON-STABILIZED SPIROKETAL ISOMERS

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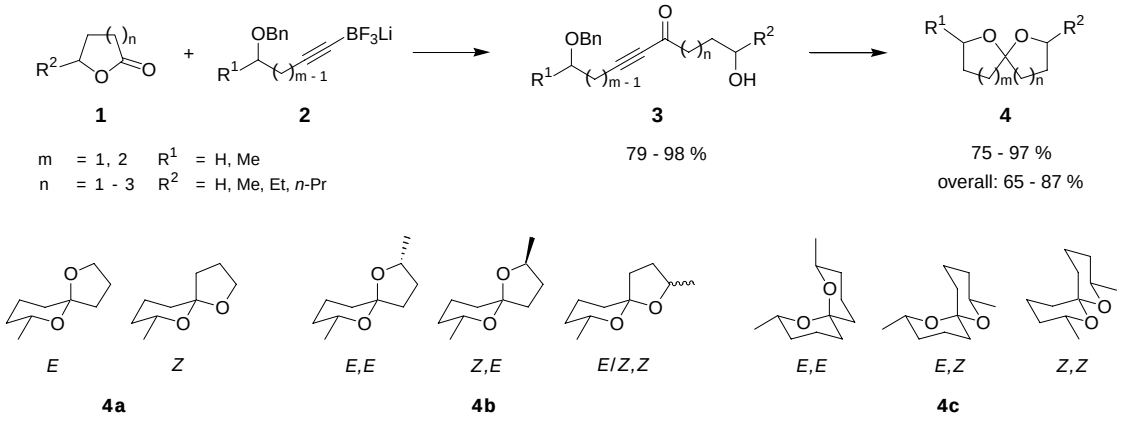
The spiroketal moiety forms a characteristic molecular element of many biologically active natural products including insect semiochemicals [1], marine and fungal toxins, polyether ionophore or macrolide antibiotics, and steroidal sapogenins [2]. Although a couple of thermodynamically less stable (*Z*)- spiroketal isomers with six-membered ring occur in nature, no general synthetic approach for the preparation of such systems was described in the literature.

Herein we wish to report a novel synthetic strategy based on the ring-opening reaction of lactones **1** with lithium alkynyltrifluoroborates **2** [3]. The benzyl-protected hydroxy-α-alkynones **3** thus obtained were found to be convertible into [4.4], [4.5], [5.5] and [5.6] spiroketals **4** by a one-pot cascade consisting of palladium-catalysed hydrogenation of the triple bond, hydroxyl group deprotection and spirocyclization under mild non-acidic conditions [4]. In comparison with the previously reported methods that start from lactones, the present route to spiroketals **4** proceeds with considerably higher yields. Moreover, the neutral conditions used in the ketalization step support the formation of less stable (*Z*)-isomers that are separable by liquid and/or gas chromatography. In this manner were, for the first time, prepared a characterized (*Z*)-isomers of **4a** and **4b** respectively, and also known (*Z*,*Z*)-isomer of **4c**.



Acknowledgements:
Financial support from the research project Z4055 905.

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NEW MACROCYCLES WITH PLANAR CHIRALITY

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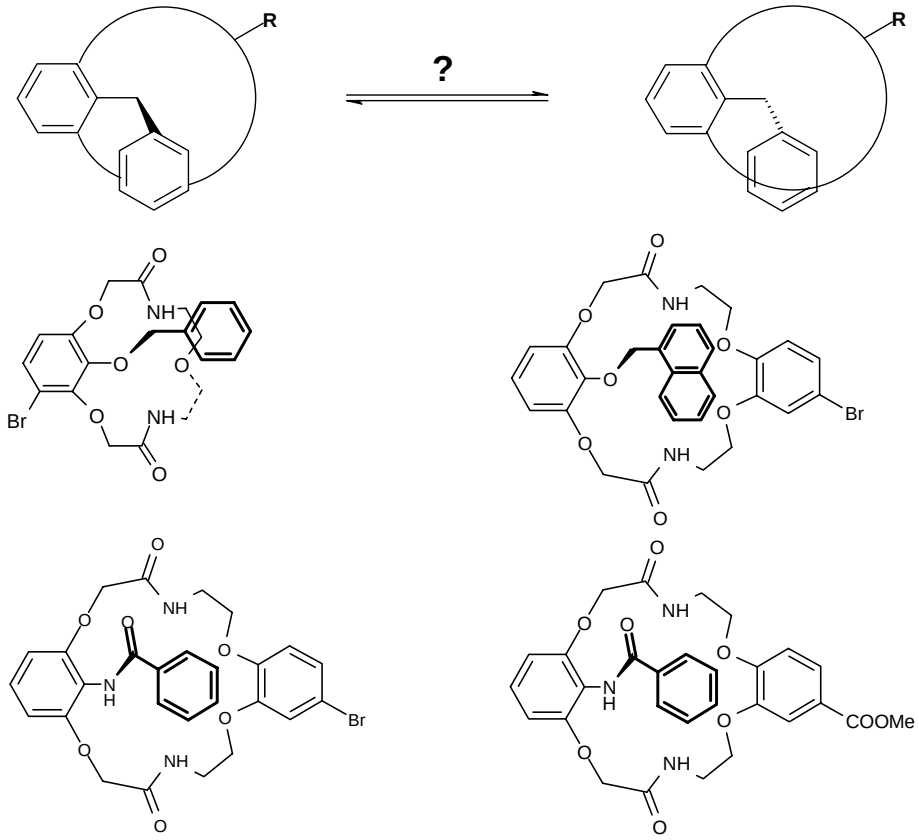
Structural studies of molecules possessing an element of planar chirality have started in 1940's with the synthesis and resolution of 1,12-dioxa[12]paracyclophane [1]. The interest in the synthesis of planar-chiral compounds has been rapidly growing since they are used as ligands for many types asymmetric reactions [2].

In this communication we would like to present the synthesis and structure elucidation of four compounds. Planar chirality of which originates from a suitably substituted macrocyclic amides.



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CHEMOENZYMATIC APPROACH TO THE SYNTHESIS OF PEPTIDOMIMETICS AS POTENTIAL ANTI-TUMOR AGENTS

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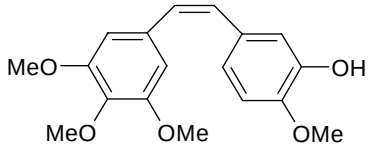
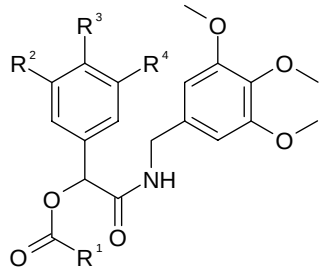
Combretastatin A-4 **1** (CA4) is a potent naturally occurring anti-mitotic agent. It's function in biological systems is based on inhibition cellular tubulin polymerization by binding to the colchicines site.[1] This compound possesses strong cytotoxicity against a variety of human cancer cells and is not subject to the multiple drug resistance phenomenon. Thus, given its favorable biological profile, many structure–activity relationships have been reported for CA4 itself in an effort to improve the natural substance [1-3].



1

Moreover, SAR studies have shown that one of the important structural features of combretastatin A-4 for binding to tubulin is the methoxy groups.

Based on this phenomenon we attempt to synthesize peptidomimetics **2** that share homology with compound **1**.



2

The general chemoenzymatic strategy employed for the stereoselective synthesis of the new peptidomimetics of general structures **2** will be presented.

This work was supported by Polish State Committee for Scientific Research, grant No. PBZ-MIN-007/P04/2003

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CHEMOENZYMATIC APPROACH TO THE SYNTHESIS OF SIMVASTATINE

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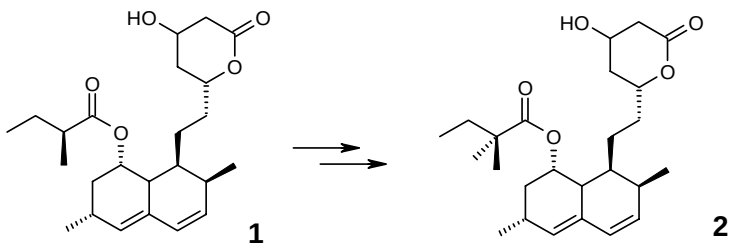
Statins are a group of antihypercholesterolemic agents. Two of them, simvastatine (**2)** and lovastatine (**1)**, have found practical use in medicine. Simvastatine is less toxic and therapeutic doses required are much lower, therefore widely used in therapies [1].



Simvastatin is synthesized from Lovastatin by C-methylation of side chain of lovastatin [2]. The separation of simvastatin from unreacted lovastatin is achieved by enzymatic hydrolysis of the later, using whole cells or isolated enzymes. Application of microorganisms brings many disadvantages e.g. difficult products separation from reaction mixture. Therefore to overcome this problem, we isolated protein fraction exhibiting lovastatin hydrolase activity. The aim of this research is to find practical way of immobilization of this enzyme in order to ensure its re-use in process. Results of the influence of the carrier and immobilization method on the enzyme activity and reactivity will be presented.

This work was supported by Polish State Committee for Scientific Research, grant no. PBZ-MIN-007/P04/2003

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EFFICIENT OLEFIN ISOMERIZATION – RING CLOSING METATHESIS REACTION IN STERICALLY HINDERED SYSTEMS. STUDY ON SIMULTANEOUS USE OF GRUBBS METATHESIS AND RUTHENIUM HYDRIDE ISOMERIZATION CATALYSTS

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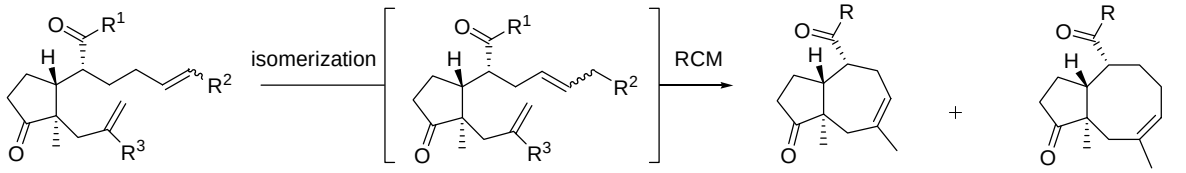
Published reports on olefin isomerization under conditions of ring closing metathesis reaction (RCM) with the use of Grubbs' ruthenium catalysts are scarce [1]. We have recently noted that certain sterically congested 1,9-diene on treatment with second generation Grubbs' catalyst **8** affords the product with a seven-membered ring (over 80% yield) instead of the expected cyclooctane derivative [2]. It was anticipated that ruthenium hydride spieces generated from **8** are responsible for the double bound shift. The aim of present work was to examine the scope of the olefin isomerization – RCM reaction and to estimate effect of ruthenium hydride catalyst [3] **9** on the RCM reaction.

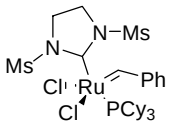
It was found that the double bond shift – RCM reaction is the dominant process for the 1,9-dienes **1**-**3**. The respective cycloheptane derivatives **4**-**6** are formed with excellent yields. The ruthenium hydride catalyst **9** proved to be compatible with the metathesis catalysts and facilitate isomerization – metathesis reaction (see on Scheme, in brackets are given yield and time of simultaneous use of mixture of catalysts **8** and **9**). Stereochemical factors favouring the formation of cycloheptane derivatives will be discussed. Additionally, efficient isomerization of an unactivated olefine with **9** will be presented.



1, R^1 = OMe, R^2 = H, R^3 = Me
2, R^1 = S^{t}Bu R^2 = H, R^3 = Me
3, R^1 = S^{t}Bu R^2 = Me, R^3 = Me

4, R = OMe 80%, 72h (94%, 0.5h)
5, R = S^{t}Bu 49%, 72h (100%, 6h)
6, R = S^{t}Bu 34% 72h (86%, 24h)

7, R = S^{t}Bu, 40%



8

$RuClH(CO)(PPh_3)_3$

9

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HYDROXYLATION OF BI- AND TRICYCLIC ENONES BY *FUSARIUM CULMORUM*

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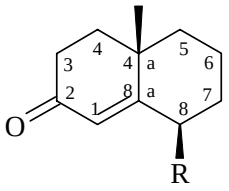
The genus *Fusarium* contains a number of species of moulds with worldwide distribution. Members of the genus have been used to metabolize natural and pharmaceutical products, especially steroids [1-5]. The main bioreaction observed in the mentioned researches was hydroxylation in different positions of steroid skeleton depending on the structure of a substrate.

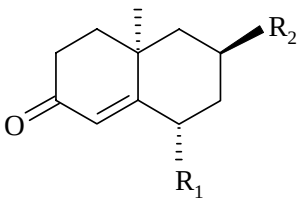
In our previous work we investigated the transformations of the 4-en-3-one steroid hormones and their derivatives by means of *F. culmorum* AM282 [4-5]. We have noticed that hydroxylations proceeded with significant regio- and stereospecifities. The results obtained indicate an influence of stereoelectronic and steric effects of substitutes on regioselectivity of the hydroxylation.

In this study, in order to explore biocatalytic potential of this microorganism, conversion of 4,4a,5,6,7,8-hexahydro-4a-methyl-2(*3*H)-naphthalenone *I* (which is a partial structure of various higher terpenes, including steroids), and its derivative 1,4,4-trimethyltricyclo[5.4.0.0^{3,5}]undec-7-en-9-one *IV* was investigated. Regio- and stereoselective hydroxylation of such compounds can provide optically pure derivatives which are often used as building blocks for further synthesis of bioactive products.

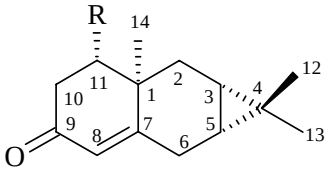
It was found that *S*-enantiomer of *I* was regioselectively hydroxylated at the axial allylic 8*R*- position, whereas transformation of the *R*-enantiomer led to mixtures of (6*S*) and (8*S*) hydroxy derivatives in 7:2 ratio. Tricyclic, sesquiterpenoid derivative of *R*-hexahydronaphtalenone was hydroxylated at the 11-equatorial position. (4a*S*,6*S*)-4,4a,5,6,7,8-Hexahydro-6-hydroxy-4a-methyl-2(3*H*)-naphthalenone *III* and (11*S*)-hydroxy-1,4,4-trimethyltricyclo[5.4.0.0^{3,5}]undec-7-en-9-one *V* were not previously reported.



S-I R=H
II R=OH



R-I R_1=R_2=H
ent-II R_1=OH, R_2=H
III R_1=H, R_2=OH



IV R=H
V R=OH

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INFLUENCE OF THE SUBSTITUTION AT C21 IN CARDENOLIDES ON THEIR BEHAVIOUR IN THE *RAYMOND* REACTION (*m*-DINITROBENZENE + NaOH)

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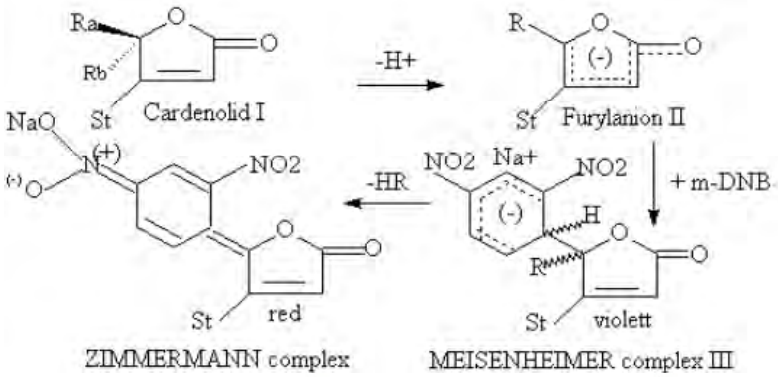
GROUP	C21 SUBSTITUTION	COLOUR	COMPLEX
	none		
A	(2H)	violet	Meisenheimer III
	Mono-substitution[x]		
B1	CH_3	violet	Meisenheimer III
B2	Br, Cl[xx]	red	Zimmermann IV
B3	F, OAc, SAc, OMe, N_3, CN, ONO_2, 14β,21(S)-epoxy[xxx]	no color	none
	Di-substitution		
C	2 Br	no color	none

[x]both epimers, [xx]only R21a = Cl = 21(R)-21-Cl, [xxx]R21b = O-C14, only this epimer.

The **RAYMOND** reaction was used for several decades to detect cardenolides (Ra,b=H,H) (group **A**) in solution or at chromatograms by development of the **violet** colour of the MEISENHEIMER complex III (R = H). This was formed after primary deprotonation at 21-CH2 by NaOH under formation of the furyl anion II followed by addition of m-dinitro-benzene (m-DNB) to III. Substitution of one H at C21 by synthesis may have different consequences for the reaction which are independent of the stereochemistry (21-R/S). Accordingly, both epimeric 21-CH_3 derivatives (group **B1**) show the same **violet** colour of the **MEISENHEIMER** complex III (R21=CH_3) as it was observed with compound A. This demonstrates that only one H atom at C21 is sufficient for the reaction. Under these conditions, the compounds of group **B2** gave a **red** colour indicating that the **ZIMMERMANN** complex IV is formed by HBr or HCl elimination. Compared to III, the π-electron system in IV is more extended. Therefore, the colour is changed to red. The compounds of group **B3** gave **no colour** under the reaction conditions despite although H at C21 is available for deprotonation. However, competing hydrolysis reaction(s) may be to fast so II cannot be formed. The compound as in **C** gave **no colour** under the reaction conditions because there is no H at C21 so that formation of a furyl anion is impossible.

St = 3β,16β*-diacetoxy-14-hydroxy-5β,14β-androstan-17β-yl; *or 16β-H, if $R21_{a/b}$ = CH_3



SYNTHESIS OF GLYCOCONJUGATE OF VITAMIN E

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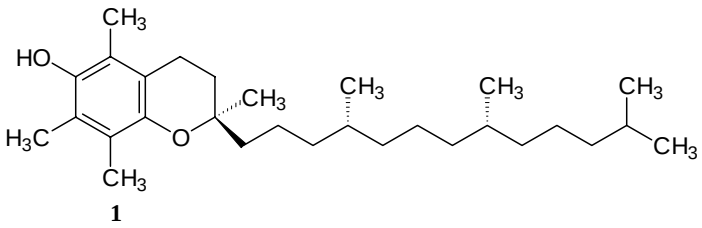
University of Białystok, Institute of Chemistry,

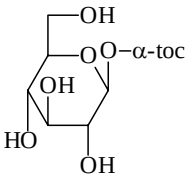
al. Piłsudskiego 11/4, 15-443 Białystok, Poland

Vitamin E (tocopherols) is a membrane-targeted scavenger of lipid peroxyl radicals. The polyisoprenoid (phytyl) side chain in vitamin E is presumably required to give the antioxidant molecule appropriate lipophilicity and solubility in membrane lipid bilayer [1]. However, therapeutic application of this vitamin is limited by its very low solubility in biological fluids. Therefore, several attempts have been made to increase the bioavailability of vitamin E. One of a possible solution is to connect lipophylic tocopherol molecule with a carbohydrate moiety [2,3]. Due to such conversion, a potential drug gains higher solubility in biological fluids as well as a better permeability through cellular membranes and biological barrier (i.e. blood-brain). Furthermore, the glycoconjugate as an amphiphylic prodrug is better distributed and more selectively transported to the injured tissues. The active tocopherol molecule can be released by action of endogenic glycosidases or acidic hydrolysis.



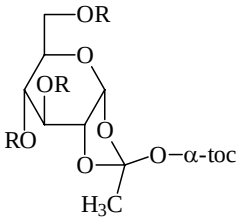
1

α-tocopherol (α-toc)



2

β-glucoside



3: R = OAc, **3a:** R = H

1,2-orthoacetate

The glycosides of α-tocopherol showed a considerable stability toward enzymatic hydrolysis [3,4]. The ortho ester derivatives of vitamine E **3a** (1,2-O-(1-tocopheroxyethylidene)-α-D-glucopyranose) seem to be more advantageous in possible application as prodrugs comparing to the glycosides **2** due to their lower stability in acidic medium.

We have found an efficient method of synthesis of sugar 1,2-orthoacetate conjugates of sterically crowded phenols. Reflux of a sugar anomeric iodide with **1** and Hunig base provided orthoacetate **3** (60-70% yield). Moreover, the anomeric iodides can be substituted by the proper anomeric bromides and chlorides, which are easier to obtain and to handle. In such a modification an addition of n-Bu_4NI was required. Removal of protective groups in a sugar moiety in **3** ($MeOH/NH_3$) gave free 1,2-orthoacetate **3a**, which appeared less stable than α-tocopherol glycoside **2**.

Investigation of stability of 1,2-orthoacetates under enzymatic and acidic conditions are in progres.

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STUDY ON ACTIVITY DATA IN QSAR MODELS. APPLICATION ON BRASSINOSTEROIDS FIELD.

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Structure-activity relationships (SAR) depend on the activity data used. This dependency is greater if quantitative structure-activity relationships (QSAR) are required. In these studies is recommended to use as activity data the logarithm of dose at 50% of highest response. Activity data must cover a minimum range of two logarithm units and must be homogeneously distributed [1]. Related to bioassay is important that the number of assays was high enough to asses reproducible results and the response had the minimum dispersion. For this reason is very difficult to get good quantitative structure-activity relationships with data obtained from literature.

Our laboratory has a wide experience in obtaining activity data in the field of brassinosteroids (BRs). The rice lamina inclination test described by Takeno and Pharis [2] was modified increasing its sensibility and specificity [3]. This has allowed to get confident activity data to develop QSAR studies. Based on GRID methodology [4] we have obtained a good model that correlates the structure of BRs with its activity expressed as minus logarithm of dose at 45º [3].

This QSAR model only considers active BRs. Those inactive or low active, with a response lower than 45º at 1 μg/pant, can not be included in the model because is not possible to determine the activity data (minus logarithm of dose at 45º). A good example to illustrate this is BRs with saturated side chain. None of them is included in QSAR model. But when they are extrapolated in the model the predicted activity values are clearly overestimated. We consider that the incorporation of this and other inactive BRs in our QSAR models would report complementary information about the structural requirements for BRs to elicit activity.

In this communication, new QSAR models considering the response at different doses which allows studying both active and inactive BRs will be presented and discussed.

Acknowledgements: This work was supported by Ministerio de Ciencia y Tecnologia of Spain (BQU2003-07852).

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PRODUCTION OF PR TOXIN, THE STARTING MATERIAL FOR THE SYNTHESIS OF KM-01 MIMETICS WITH BRASSINOSTEROID ACTIVITY

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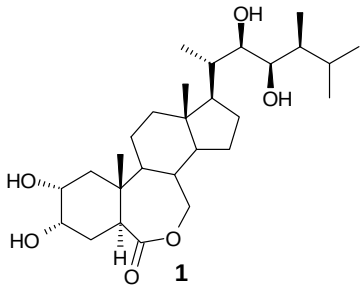
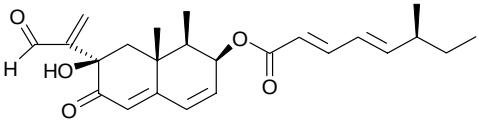
Brassinosteroids (BRs) are steroidal phytohormones that show remarkable plant physiological activities, improving the crop yields and increasing tolerance to stress due to temperature, water or salinity [1]. However, their synthesis or isolation from plants is really time consuming and expensive. So, we are focussing some efforts in the design and synthesis of new non-steroidal mimetics to better understand their mode of action in plants. In this field, our group compared the properties and structural characteristics between brasssinolide (**1**) and the antagonist KM-01 (**2**) [2]. This study allowed determining their structural similarities and the specific groups for each one.

By means of molecular modeling techniques we have designed a new family of KM-01 (**2**) analogues which has the groups required to elicit BR activity. For the synthesis of these mimetics, it is necessary the production of PR toxin (**3**) to use it as starting material. So, base on Wei and Chang works [3,4], we have improved their methodology for obtaining this metabolic product.

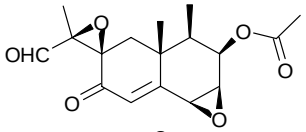
In this communication, we will present the improvement made on the Wei [3] and Chang [4] methodology for the production of PR toxin (**3**) by *Penicillium roquefortii*.



1



2



3

Acknowledgements: This work was supported by a grant (IJ) from Generalitat de Catalunya (DURSI, Nº 2002FI 00701) and European Social Fund. Moreover, we want to thank Ministerio de Ciencia y Tecnologia of Spain for supporting that project (BQU2003-07852).

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3D-QSAR MODEL STUDIES FOR BRASSINOSTEROIDS USING GRID INDEPENDENT DESCRIPTORS (GRIND)

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The application of brassinosteroids in improving the yield and quality of crops has attracted attention in these natural plant hormones [1].

In order to achieve a more efficient application of these compounds, studies about the relationship between structure and activity have been carried out. They are focused in the structural requirements needed for a compound to elicit brassinosteroid activity. This allows us to know which peculiarities share brassinosteroids and provides us more information about the mechanism of action of such compounds.

With this aim, some 3D-QSAR models have been developed in our group using the GRID methodology that requires the previous alignment of the compounds [2]. From the information given by these models, we pointed out the importance of hydrogen bonding interaction between brassinosteroids and the putative receptor.

Recently, it was found that brassinosteroids bind to the extracellular domain of BRI1, a receptor serine/threonine kinase localized on the plasma membrane of plant cells [3]. The crystallographic structure of this protein is still unavailable. Therefore, the further QSAR studies must be still indirect, that is, considering only the structural features of the substrate.

In order to obtain complementary information, we have put into effect 3D-QSAR studies made with GRID Independent Descriptors (GRIND) [4], computed with the program ALMOND [5]. These recently developed descriptors are based in distances between different regions of potential interaction in the molecule avoiding the alignment of the compounds. In this communication, we present the obtained results, which help us to know more about the way of action of brassinosteroids.

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NEW BRASSINOSTEROID DERIVATIVES AT THE C2 POSITION OF THE A RING. APPROACH TO THE TYPE OF BRASSINOSTEROID-RECEPTOR INTERACTION

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Brassinosteroids are a kind of plant growth regulators widely distributed in the plant kingdom[1].

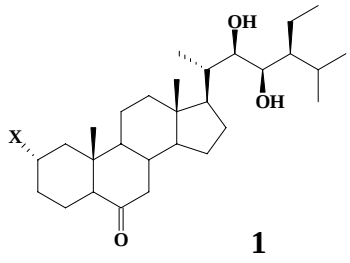
Since the discovery of brassinolide, which shows a high activity as a growth promoter, many studies have been focused on the synthesis of new analogs for a better understanding the mode of action of such compounds.

In our aim in obtaining information about the structural requirements for a brassinosteroid to be active in an efficient way, a quantitative structure-activity relationship (QSAR) has been developed in our group based on molecular modeling techniques. Our results confirm that electrostatic charges play an important role in displaying biological activity. Moreover, we have found that hydrogen-bonding could be one of the types of interactions that could be taking place upon binding [2].

The assessment of H-bonding and the clarification of how the functionalities present in a brassinosteroid could work on binding through hydrogen bonding with the receptor has become a priority aim in our group [3].

In this communication the synthetic strategy developed to obtain new brassinosteroid analogs having other functionalities than hydroxyl at C2, which can act as acceptor or donor of hydrogen bonding will be presented as well as their activity data.

Moreover, how the 2α-OH of a brassinosteroid works through hydrogen bonding with the receptor will be specifically analyzed.



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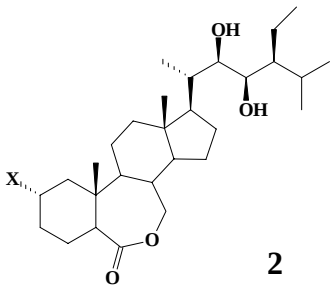

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EVALUATION OF BIOLOGICAL ACTIVITY OF ANDROSTANE BRASSINOSTEROIDS WITH 17β-ESTER GROUPS

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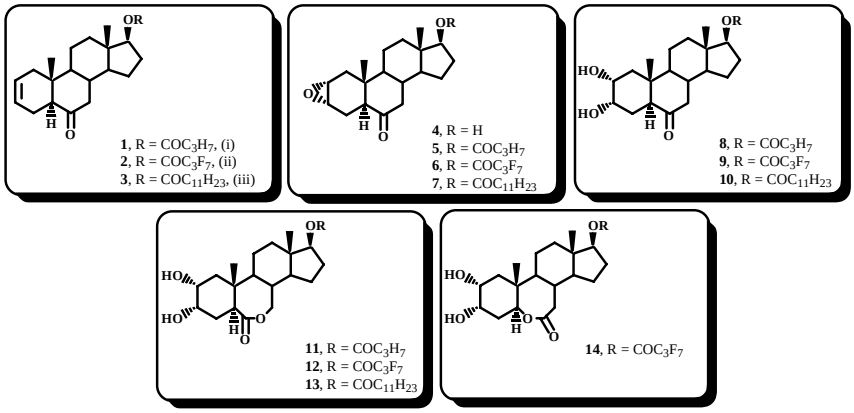
As continuation of our structure-activity relationship studies and also activity-cost relationship of brassinosteroids we synthesized the brassinolide analogues of 17β-esters derivatives of androstane to partly simulate the polar part of brassinolide side chain replacing the complicated C-C formation by easier esterification. During our studies series of brassinosteroids were prepared and some of them showed remarkable activities. One of these groups was androstane analogues of brassinolide with ester group with five carbon atoms in the position 17β [1,2]. As these esters showed high activity, we decided to synthesize similar compounds with various ester groups, with short chain corresponding to butyric acid, and long chain corresponding to dodecanoic acid, and full fluorinated chain corresponding to heptafluorobutyric acid [3].

There are only few RLIT (Rice Lamina Inclination Test) results but, they do not correspond with BSIB (Bean Second Internode Bioassay) results. Compounds **8**, **10**, **11** and **13** are inactive on RLIT but on BSIB exhibit some activity. High activity was found also at heptafluorobutyrate **6** and laurate **13**. Bioassays on the field conditions are in progress.



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2,3-DIOLS VERSUS 3,4-DIOLS IN THE BEAN SECOND INTERNODE BIOASSAY

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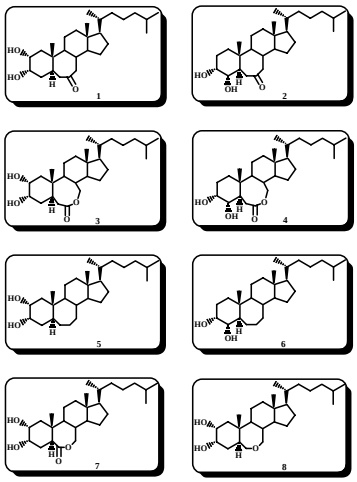
Brassinosteroids, a class of potent plant growth regulators, have a promise of potential use in agriculture due to their ability to improve crop yields, overcome environmental stress [1] and being environmentally friendly. Having studied [3-5] the structure-activity and cost-activity relationship of brassinosteroids, we now turn our attention to 7a-homo- and 7a,7b-dihomo-5α-cholestane [5] analogues of brassinolide. These products were employed in our screening project and their activities were determined by the modified BSIB (Bean Second Internode Bioassay) [6]. We have found that, the 3α,4α-diols **2**, **4** and **6** are more active then the 2α,3α-diol **1**, **3** and **5**. It could be explained by twisting and distortion of the molecule due to seven or eight membered B-ring and also by distance of two active binding zones (vicinal diol and carbonyl or lactone) to receptor. In addition, the influence of carbonyl group on biological activity was examined. In the case of compound **8** without carbonyl group decreased activity is observed in comparison with compound **7**.

Elongation (mm)	Applied amount (M)					
of compounds	10^{-12}	10^{-11}	10^{-10}	10^{-09}	10^{-08}	10^{-07}
24-epiBL	5.5	23.9	58.1	58.5	29.5	
1	2.1	3.7	-3.2	**2.8**	3.3	3.8
2	2.4	8.6	19.5	**22.5**	14.2	3.9
3	0.6	7.3	17.2	**9.0**	10.9	2.2
4	0.2	6.2	6.9	**20.5**	9.3	16.7
5	5.8	9.0	7.3	**2.5**	4.1	-7.7
6	5.1	3.8	18.0	**11.9**	8.5	20.1
7	2.0	5.2	8.5	**13.8**	8.9	3.7
8	4.7	8.4	1.2	**3.4**	4.3	2.7



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DETERMINATION OF ENDOGENOUS BRASSINOSTEROIDS BY IMMUNOAFFINITY CHROMATOGRAPHY AND HPLC/MS

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Brassinosteroids (BRs) are polyhydroxylated steroid plant hormones showing essential effects on growth and development of plants. They induce cell elongation and cell division, increase DNA and RNA polymerase activity; interact synergistically with auxins; stimulate ethylene production, increase tolerance to stress due to temperature, water or salinity. The detection and quantification of brassinosteroids is quite difficult because its amount in plant tissues is extremely low. We have established sensitive and specific method for brassinosteroid determination based on combined immunoaffinity chromatography and HPLC/MS.

We have developed polyclonal antibodies against a brassinosteroid analogue, (20S)-2α,3α-dihydroxy-7-oxa-7α-homo-5α-pregnane-6one-20 carboxylic acid (4812). Antiserum against this substance was raised in rabbits immunized using 4812 - 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 (20S)-2α,3α-dihydroxy-7-oxa-7α-homo-5α-pregnane-6one-20 carboxylic acid – horse-radish peroxidase conjugate. The cross-reactivities of 11 compounds including the natural and synthetic BRs were investigated, revealing the broad specificities of the antibodies.

After immunopurified samples were analyzed by high performance liquid chromatography (Waters; model Alliance 2690) linked to a Micromass ZMD mass spectrometer equipped with electrospray interface (LC (+)ESI-MS). The samples were injected on a C18 reversed-phase column (Phenomenex, Gemini 50 x 2.0 mm, 5 micron). The following solvents were used: MeOH (solvent A), 5 mM formic acid (solvent B), and the column was eluted with 70% A-30%B for 10 min, flow 0.6mL/min.

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IMMUNOANALYSIS OF STEROID HORMONES IN PLANTS

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The occurrence of large quantity of steroid compounds in plants is recently quite well documented. There are many classes of phytosteroids which have been isolated from different plant sources, like brassinosteroids, bufadienolides, cardenolides, cucurbitacins, ecdysteroids, steroidal saponins, steroidal alkaloids, withanolides and also vertebrate-type steroids [3], which we are interested in. Indirect evidence of vertebrate-type steroids occurrence was given by studies using labeled sterols entering metabolic pathways in plants [1, 2]. The wide array of steroids which figure as hormones in vertebrates was gradually also isolated from different plant species [3, 4]. Although it is known, that many of these structures occur in plants naturally, their role is still uncertain [5, 6]. The aim of our work is to develop an effective method for isolation and identification of these steroids from plants.

We have developed polyclonal generic antibodies against steroid structures using dehydroepiandrosterone–17-carboxymethyloxime and 4-androstene-3-one-17-carboxymethyloxime conjugated with BSA. Both antigens led to preparation of high affinity antibodies giving very high sensitivity in ELISA. The measurement of cross-reactivity with large portfolio of steroid structures confirmed expected generic character of these antibodies. The antibodies will be used for development of an immunoaffinity chromatography combined with HPLC-MS.

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EFFECT OF PLANT TERPENOID FETUTININE ON PERMEABILITY TRANSITION IN RAT LIVER MITOCHONDRIA

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Previously we found that esters of sesquiterpenic alcohols with aromatic but not aliphatic acids isolated from the plant of the genus Ferula increase the cation permeability of lipid bilayers and mitochondria. One of them, phytoestrogen Ferutinine displayed the highest activity as electrogenic Ca-ionophore [1]. Further studies of Ca-ionophoric properties of Ferutinin on isolated cells (neurons and hepatocytes) demonstrated, that the increase of cytosolic Ca^{2+} is accompanied by accumulation of Ca^{2+} in mitochondria. This was followed with depolarization of mitochondria, which could be prevented by cyclosporine A, an inhibitor of mitochondrial pore. However in the absence of external Ca^{2+} and in the cells loaded with BAPTA-2AM, Ferutinine also induced depolarization of mitochondria, although to a lesser extent [2].

The present study was performed to examine the mechanisms of Ferutinine action on the permeability transition pore (PTP) in mitochondria isolated from rat liver. The opening of PTP was determined as swelling of non-energized mitochondria in sucrose containing medium. It was shown that Ferutinine increased the effect of Ca^{2+} ions in the range of concentrations 1-50 μM. Addition of cyclosporine A resulted in a considerable (5 fold) decrease of both the rate and the amplitude of Ferutinine induced swelling of mitochondria. Ca-ionophoric properties of Ferutinine are thought to contribute to Ca^{2+} accumulation in mitochondria and, as a consequence, to the opening of PTP into a highly conductive state. However, using a prooxidant cumole hydroperoxide as an inductor of PTP we found that Ferutinine increases an cumole hydroperoxide influence on permeability of mitochondrial membranes under similar experimental conditions. The effect of Ferutinine in the latter case was, however, lower than when used as an inductor of Ca^{2+} ions. Cyclosporine A inhibited ferutinine+ cumole hydroperoxide-induced permeabilization of mitochondria completely. In the absence of a pore inductor, ferutinine did not induce marked changes in membrane permeability of non-energized mitochondria over the range of concentrations. We conclude that the effect of Ferutinine on PTP in mitochondria can not be explained either by the detergent-like properties, or by its Ca ionophore properties alone.

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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF VOLATILES FROM FLOWERHEADS OF *CENTAUREA POLYMORPHA* LAG. (ASTERACEAE)

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The genus *Centaurea* belongs to the Cynareae tribe of the Asteraceae family and comprises annual, biennal and perennial grassy plants. Taxonomically, the genus is highly polymorphous and previous studies on the roots and green parts of several *Centaurea* have shown the presence of numerous secondary metabolites [1-5]. Recently, we reported on the volatile components of some *Centaurea* species growing wild in Sicily [6] and Lebanon [7].

In this paper we report on the volatile components of the flower-heads of *Centaurea polymorpha* Lag. wild growing in Spain. At the best of our knowledge, no analyses have been previously reported on this plant.

The flower-heads were collected at the full flowering period. from plants grown at Cogolludo, near Aliendre river (Spain). The air-dried samples were crushed and subjected to hydrodistillation as previously described [8]. GC, GC/MS analyses and biological activity evaluation against eight eight selected Gram positive and Gram negative bacteria were performed as previously described [8].

Sixty three compounds, representing 91.6% of the oil, were identified. The main compounds are distributed within saturated hydrocarbons, sesquiterpenes and fatty acids. The main sesquiterpenes were α-cedrene (3.9%), β-cedrene (3.6%), β-curcumene (3.0%) and caryophyllene oxide (2.6%).

No biological activity on Gram positive and Gram negative bacteria was detected.

Acknowledgments: GC/MS spectra were performed at the "C.S.I.A.S.", University "Federico II", Napoli. Research was supported by a grant of the Regione Campania.

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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS FROM AERIAL PARTS OF TWO *MARRUBIUM* SP. (LAMIACEAE) GROWING WILD IN LEBANON

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The genus *Marrubium* includes about 40 species, indigenous in the Mediterranean area, Europe and Asia [1]. Many *Marrubium* species are reported in the literature to be used in traditional medicine for treating a variety of diseases and for their neurosedative and anti-inflammatory activities [2]. Aerial parts of *Marrubium* sp. contains flavonoids [3], phenyl ethanoid glycosides [4], phenylpropanoids and several labdane diterpenoids [5]. The oils of *Marrubium* species have been the subject of a few investigations [6,7].

Now we report the analysis of the essential oils of *M. globosum* Montbr. et Auch. ex Benth. ssp. *libanoticum* (Boiss) Davis and *M. cuneatum* Banks et Solander that grow wild in Lebanon. The aerial parts of plants were collected in Lebanon at the full flowering period. GC and GC/MS analyses and evaluation *in vitro* with paper-disk diffusion method against eight selected Gram+ and Gram- bacteria were performed as previously described [8].

Altogether 64 compounds, representing 93.4% and 91.4% of the oils, were identified. The main components of both oils were β-caryophyllene, palmitic acid and spathulenol. Bicyclogermacrene was present only in the oil of *M. cuneatum* characterized by high amount of germacrene D. Oils showed little antimicrobial activity.

Acknowledgments: GC/MS spectra were performed at the "C.S.I.A.S.", University "Federico II", Napoli. Research was supported by a grant of the Regione Campania.

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NEW DITERPENES FROM *MARRUBIUM GLOBOSUM* MONTBR. ET AUCH. EX BENTH. SSP. *LIBANOTICUM* BOISS. (LAMIACEAE)

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The genus *Marrubium* (Lamiaceae) comprises around 40 species, indigenous in the Mediterranean area, Europe and Asia [1]. Aqueous and hydroalcoholic extracts of *Marrubium* spp. are used in traditional medicine for the treatment of a number of diseases, including those affecting the gastrointestinal tract and breathing apparatus [2]. As chemical constituents of *Marrubium* sp. flavonoids, flavonoid glycosides, phenylethanoid and phenylpropanoid glycosides have been reported [3], but the main chemical feature of the genus is the presence of furolabdane diterpenes and related compounds [4], which have been isolated from almost all the species studied till now. In this communication we describe the isolation and identification of several new diterpenic compounds from the acetonic extract of *Marrubium globosum* Montbr. et Auch. ex Benth. ssp. *libanoticum* Boiss. (Lamiaceae). Although many contributions concerning the chemistry of numerous species of *Marrubium* have been reported, no phytochemical studies have been performed on the title plant; only in another subspecies, *M. globosum* ssp. *globosum* collected in Turkey, labdane diterpenes have been detected in the methanolic extract [5]. For our study, plant material (550 g) was collected in August from flowering plants from Lebanon. The powdered plant was extracted with Me_2CO and the residue was chromatographed on Si gel eluting with petrol and petrol-EtOAc. The further purification by HPLC gave pure new natural furolabdane diterpenoids. The structures of these new compounds were elucidated by spectral methods (IR, UV, MS, NMR).

Acknowledgements: Thanks are due to the C.S.I.A.S for technical assistance.

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BIOLOGICAL ACTIVITIES OF NATURAL GUAIANOLIDES FROM *CENTAUREA HOLOLEUCA BOISS* AND THEIR DERIVATIVES

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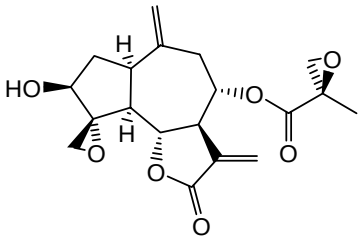
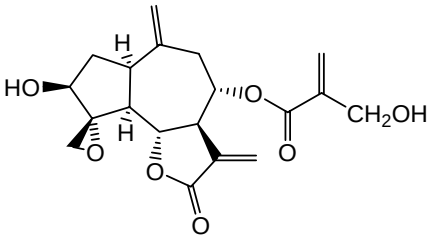
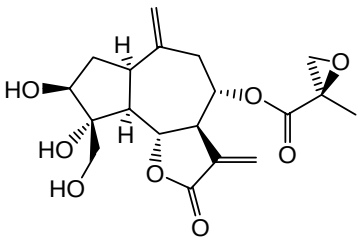
The *Centaurea* genus is a rich source of secondary metabolites such as sesquiterpene lactones [1]. The biological properties of these compounds are well-known; the most important being essentially cytotoxic activity [2]. *Centaurea hololeuca* Boiss. is a plant occurring in Lebanon. The chromatography of acetonic extract afforded several guaianolides mainly janerin [3], repin [4] and babilin B [5], having an oxirane ring and other guaianolides.



repin



janerin



babylin B

The natural compounds and some of their derivatives were tested for their cytotoxic activity and for their antifeedant activity. The results are presented. In literature there are few information about the antifeedant activity of sesquiterpene lactones, therefore the results are worth of mention.

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LIGNANS AND AN UNUSUAL STEROID FROM THE SEEDS OF *CENTAUREA SCLEROLEPIS* BOISS.

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[2] Dipartimento Chimica Organica, Università di Palermo, Palermo, Italy;

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The genus *Centaurea* L., (Asteraceae), comprises *ca.* 600 species distributed in Asia, Europa, North Africa and America [1]. Turkish flora numbers 187 species, 114 of which being endemic [2-4].

Previous chemical studies seem to indicate that sesquiterpene lactone patterns are systematically important within the genus *Centaurea*. Other metabolites present in this genus include triterpenes, steroids, hydrocarbons, polyacetilenes, flavonoids, anthocyanins, lignans and alkaloids [5]. As part of our ongoing studies on *Centaurea* species of Mediterranean area [6-9] we describe, in the present paper, the results of a phytochemical investigation on the seeds of *Centaurea sclerolepis* Boiss. (section Cyanaroides), an endemic species distributed in Eastern Anatolian regions of Turkey.

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ANOMERIC HYDROPEROXIDES DERIVED FROM 2-DEOXY SUGARS; SYNTHESIS, ENANTIOSELECTIVE EPOXIDATION

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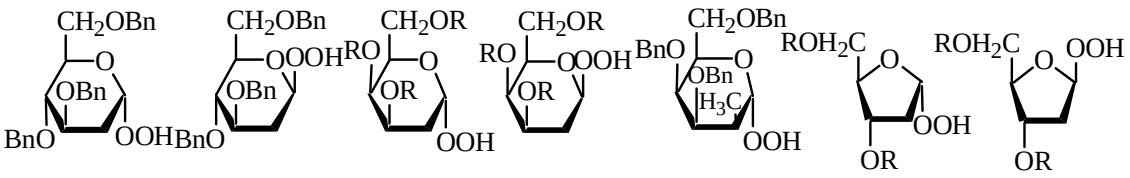
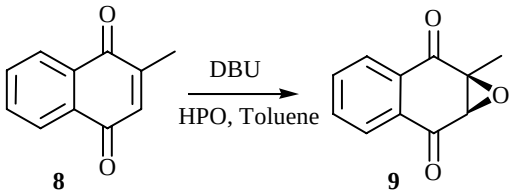
Oxidation of readily available 2-deoxysugars or their methyl glycosides with 50 % hydrogen peroxide in dioxane in the presence of sulfuric acid[1,2] provides corresponding hydroperoxides **1**-**7** in 48-75 % yields. They are relatively stable and can be separated into pure anomers by chromatography; compounds **3** and **5** practically exist as single anomers only.



1 **2** **3**: R=Bn, Piv **4**: R=Bn, Piv **5** **6**: R=Ac,Bn, Piv **7**: R=Ac,Bn, Piv

Experiments with the use of anomeric hydroperoxides **1**-**7** as chiral oxidants were performed using 2-methyl-1,4-naphtoquinone (**8**) under standard conditions provided by Taylor et al.[1,2] to afford the corresponding epoxyquinone **9** with e.e in the range 28-48%. After asymmetric epoxidation of an electrophilic olefin, the hemiacetal can be regenerated from the post reaction mixture and reoxidized again to the starting hydroperoxide.



8 **9**

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A DOUBLE ASYMMETRIC INDUCTION IN 1,3-DIPOLAR CYCLOADDITION OF CYCLIC NITRONES DERIVED FROM MALIC ACID AND TARTARIC ACID WITH UNSATURATED γ-LACTONES

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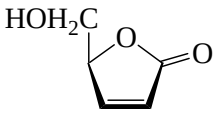
1,3-Dipolar cycloaddition of lactones **1** and **2** with nitrones **3** - **6** provides adducts **7-10**.[1] In the case of the lactone **1** and nitrone **3**/**5** only one *exo* adduct was formed, respectively, as a result of the *exo* addition, *anti* to *t*-butoxyl at C-3 of the dipole. In the case of lactone **2** and nitrone **3** (matched pairs) one stereoisomer is formed, as well. On the other hand, the nitrones **4-6** with lactone **2** (mismatched pairs) affords corresponding *exo* adducts which are accompanied by *endo* ones. This result should be compared with the same reactions performed on δ-lactones. In all those, so far, investigated cases, the formation of *endo* adducts was not observed.

1



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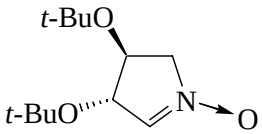

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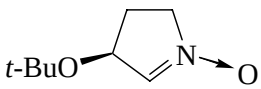

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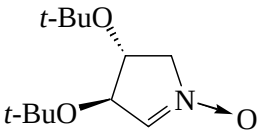
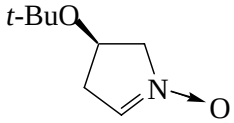
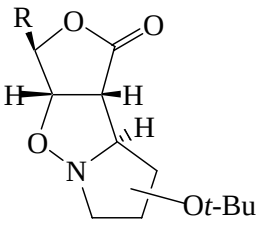


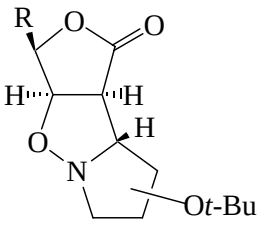
5



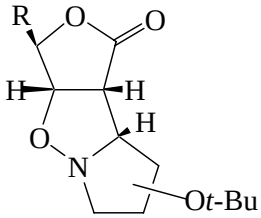
6



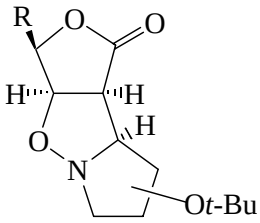
7, *exo-anti*



8, *exo-syn*



9, *endo-anti*



10, *endo-syn*

R= H, CH_2OH

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SYNTHESIS OF 8-HOMOCASTANOSPERMINE AND 7-HOMOAUSTRALINE FROM D-*TREO*-HEXALDONO-1,5-LACTONE AND A CYCLIC NITRONE DERIVED FROM MALIC ACID

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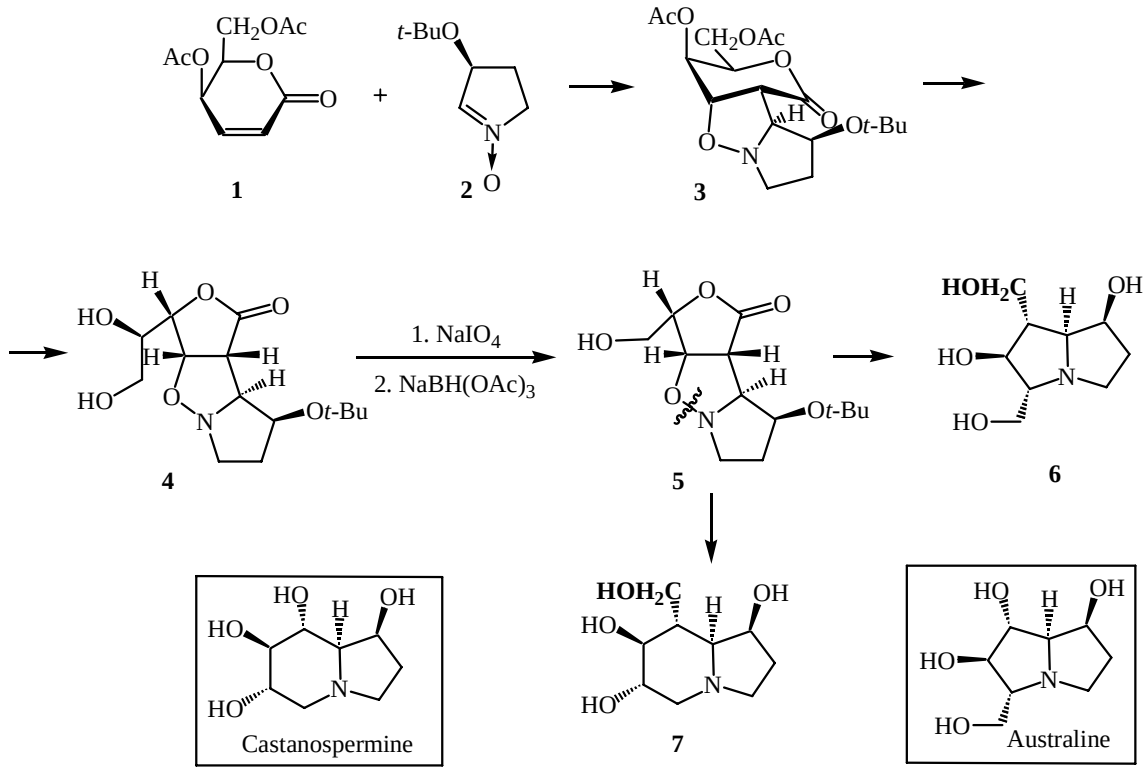
1,3-Dipolar cycloaddition of D-*threo* lactone **1** and nitrone **2** afforded a single adduct **3**.[1] Conversion of the δ-lactone fragment into γ-lactone gave **4**. Subsequent removal of the terminal atom of the sugar backbone provided a tricyclic compound **5**, which was used as attractive starting material for the synthesis of 7-homoaustraline **6** and 8-homocastanospermine **7**.



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STUDIES TOWARDS SOLID-PHASE SYNTHESIS OF NORDIHYDRODARLINGINE AND NORCHALCOSTROBAMINE

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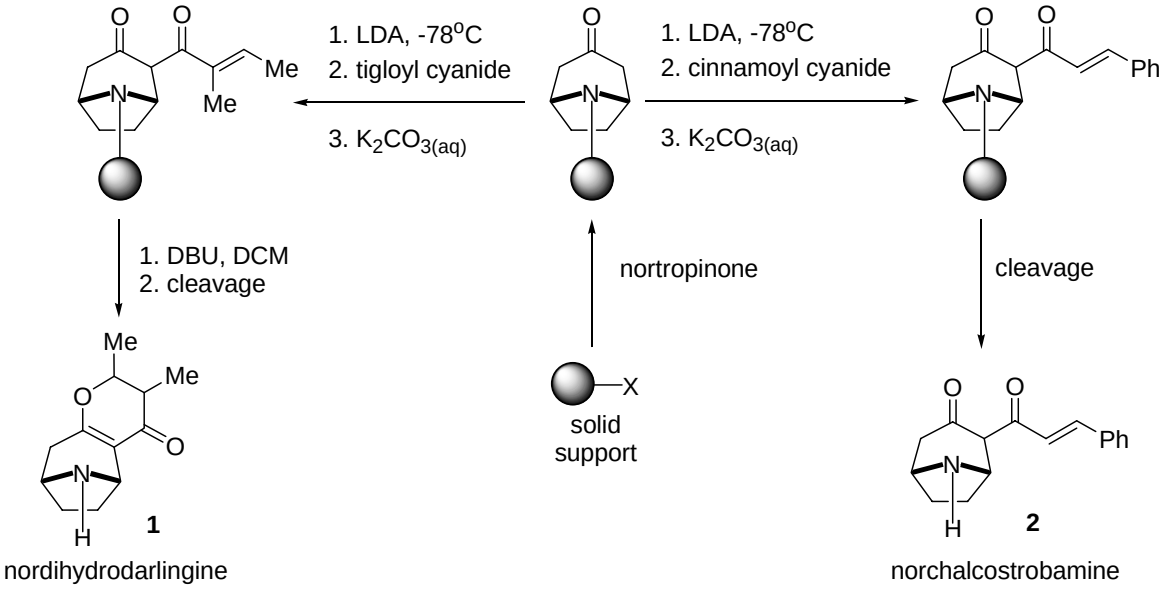
Darlingine and chalcostrobaminbe are two tropane alkaloids, which can be synthesised in racemic and enantiomerically pure form through transformation of tropane derivatives [1]. In order to elaborate related solid-phase synthesese of these alkaloids and their nor analogues (**1** and **2**) it is necessary to develop reliable methods for reversible anchoring of nortropinone on a polymeric support. This study concerns anchoring of nortropinone (8-azabicyclo[3.2.1]octan-3-one), on polymeric supports (Merrifield gel, Wang gel, 4-nitrophenylcarbonate Wang gel, trityl chloride gel, *para*-C_3-T2 triazene gel, BAL type gel and polymers with linkers analogous to BOM and MOM protecting groups). The "bind and release" process is effective on Merrifield, 4-nitrophenylcarbonate Wang, trityl chloride and *para*-C_3-T2 triazene [2] polymers. The anchored ketone is subjected to deprotonation with LDA follow by reaction with a suitable acyl cyanide. The resulting polymer bound products are cleaved from the polymeric support.



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