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18th CONFERENCE ON ISOPRENOIDS

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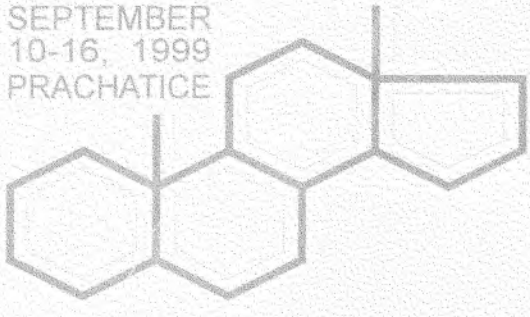

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Institute of Organic Chemistry and Biochemistry
Academy of Sciences of the Czech Republic

18th Conference on Isoprenoids

September 10–16, 1999
Prachatice, Czech Republic

Abstracts

Valterová Irena, Drašar Pavel, Pouzar Vladimír
(Issue Editors)

PREFACE

Tradition is a category that is usually damned by a Homo novus without any tradition and chanted by those who can boast about it.

The conference on isoprenoids, whose abstracts are collected in this volume, has a really respectable tradition: the first conference of the series happened 33 years ago in a little village in Poland. Two personalities – Prof. Marian Kocór and Prof. František Šorm – then came together and decided to launch a biennial tradition of scientific contacts of Polish and Czechoslovak scientists working in steroid chemistry. Each side had to organize a joint conference on its soil every four years. Highly valued scientists from the West were invited to lecture on important issues. Among the lecturers we could find names like D. H. R. Barton, G. Snatzke, I. V. Torgov, D. Arigoni, G. Ourisson, K. Nakanishi, K. Wiesner, A. J. Birch, K. Mori, G. Stork etc. Their presence opened the door to participants from other countries and soon the audience became fully international.

Topics covered by lectures ranged from total synthesis, functionalisation of an inactive skeleton to biotransformation of steroids. Various fashions of the steroid chemistry, as it developed over the years, were reflected in titles of the lectures. The scope was widened to include related topics and thus chemistry of other isoprenoids, in particular terpenoids, was included. Reflecting the biological importance of many isoprenoids, more targeted research (medicinally, agriculturally) has gained significance. It was considered as essential that topics represent the scope of isoprenoid research, including different aspects of chemistry (synthetic, analytical), biochemistry, and even molecular biology, so that different kinds of isoprenoid researchers could meet each other. The need of exchange of information among disciplines like chemistry, medicine, plant sciences, entomology and zoology is now probably greater than ever. Also it is important that biologists making the increased use of chemical techniques get a chance to provide feedback on how chemistry might be even better applied to particular problems involving biological activity of natural products.

The 18th conference in the series will come through in the Czech Republic this year. The tradition did not collapse with the fall of the Iron Curtain when traditional participants could choose from much wider an option and new quality was added to the previous standard.

At the 18th Conference on Isoprenoids 21 plenary lectures and more as 60 poster communications will be presented. We would like to express our thanks to the Czech Chemical Society which enabled us to publish abstracts of lectures and communications in its journal Chemické Listy.

We hope that all participants will find this meeting useful and will enjoy stay in Czech Republic.

June 1999

L. Kohout
A. Kasal
B. Koutek

INDUCED BIOSYNTHESIS OF TERPENOID INSECT SEMIOCHEMICALS IN PLANTS

WILHELM BOLAND, JÜRGEN ENGELBERTH, JÖRN PIEL, VERENA JUNG, ANDREAS JUX, THOMAS KOCH, THOMAS KRUMM, and GÖDE SCHÜLER

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In nature volatiles have a multitude of functions. They may act as antibiotics, fungicides, deterrents, attractants, or, more generally speaking, as INFOchemicals transferring all kind of information into a complex network of mutually interacting organisms. Plants under attack by a herbivore or micro-organism may emit characteristic volatiles that are *inter alia* implicated in the attraction of the natural enemies of the herbivore[1]. To understand the events between primary leaf damage and the emission of volatiles from the damaged plants, our research activities focus on the following topics.

1. Early events involve the interaction of low- and high-molecular elicitors of the attacking organisms with the plant "leaf-biochemistry" and require the identification of high- and low-molecular elicitors from insect herbivores and micro-organisms.

2. The second complex comprises the elements of signal transduction linking the primary events and the currently known end product (jasmonic acid) of the signalling pathway and addresses the identification of the different pathways as well as the signalling-quality of individual compounds within the signalling-pathways.

3. Recognition of the signal transducer(s) by cellular receptors and the subsequent events leading to reprogramming the gene expression of the leaf.

4. Enzymatic and regulatory aspects (resource mobilisation, enzyme mechanisms) of volatile biosynthesis.

(1) Cellulysin, a cocktail of endoglucanases and cellulases, produced by the plant parasitic fungus *Trichoderma viride* was identified as a powerful and generally active high-molecular elicitor of volatile biosynthesis in plants (*Phaseolus lunatus*, *Nicotiana plumbaginifolia*, *Zea mays*)[2]. It was found to stimulate the emission of a burst ethylene (3–6 h after stimulation) followed by the release other volatiles (fatty acid metabolites, terpenoids, aromatics) as a later event. In the Lima bean, used as the model system for most of these studies, their emission is not continuous, but follows an endogenous, circadian rhythm. Among the elicitors from oral secretions of insect herbivores (Noctuidae) amino acid conjugates of saturated and unsaturated C_{18} and C_{16} fatty acids were found to be widespread, but the compounds showed limited activity as elicitors for the induction of volatile biosynthesis in test plants.

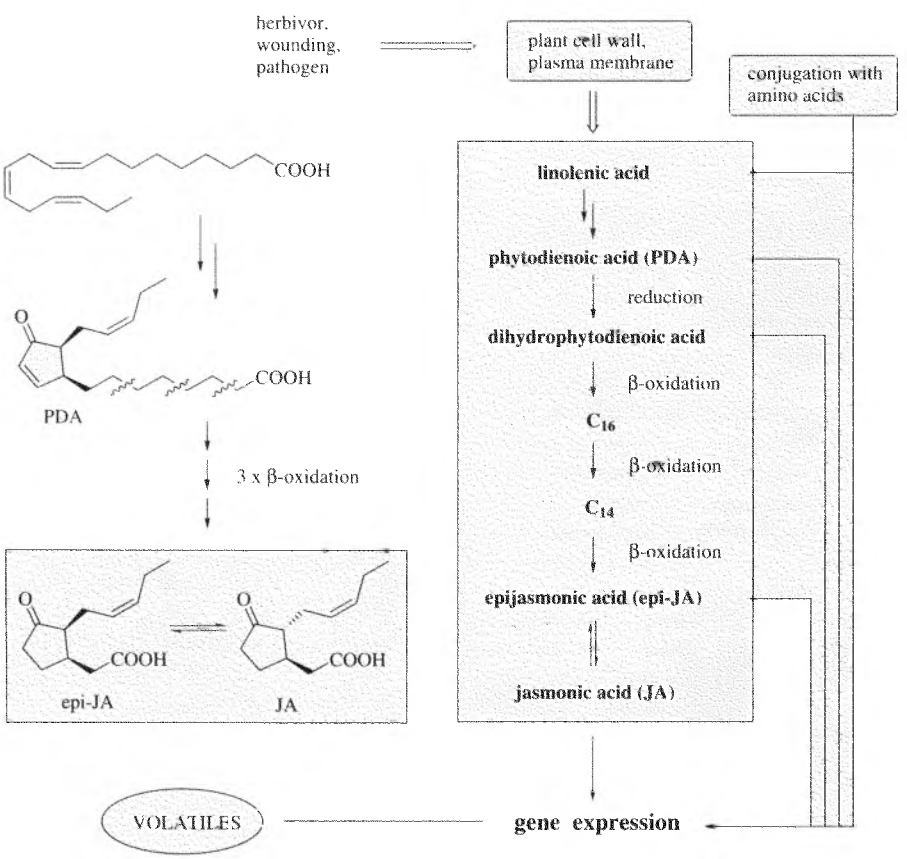

Fig. 1 Octadecanoid signalling pathway in plants

(2) Although the primary events of the leaf-cellulysin--interaction are unknown, it could be shown that the high-molecular elicitor acts via the octadecanoid signalling pathway. Inhibitors preventing lipidperoxidation like phenidone completely blocked the cellulysin-dependent volatile biosynthesis[2]. The upregulation of the JA-pathway could be also directly demonstrated by analysing the time course of the JA-level in cellulysin-treated plants[3]. Analysis of the inducing-power of individual intermediates of the JA-pathway revealed that in the Lima bean exist at least two centres of biologically active compounds. Early intermediates (linolenic acid → 12-oxo-phytodienoic acid (PDA)) induce the biosynthesis of the two homoterpenes 4,11-dimethylnona-1,3,7-triene and 4,8,12-trimethyltrideca-1,3,7,11-tetraene, representing degradation products of sesquiterpenoid and diterpenoid precursors[3]. By using inhibitors, PDA was identified as the active compound in this part of the pathway. (cf. Fig. 1). Subsequent intermediates of the pathway were synthesized (10,11-dihydro-PDA and the $C_{17} \rightarrow C_{13}$ analogues of JA) but failed to induce volatile biosynthesis[4]. JA, the last member of the octadecanoid pathway turned out to be generally active. Modern (dicots, monocts) and evolutionary ancient plants (ferns, Gingko) respond to JA-treatment with the emission of volatiles. Some plants produce novel compounds, others only respond with quantitative shifts within their blends[3].

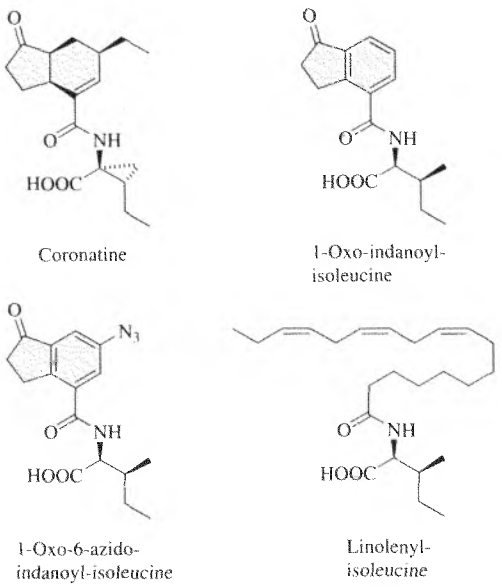

Fig. 2 Low molecular elicitors from microorganisms (coronatine) and insects (LIN-Gln) and some synthetic analogues.

Besides compounds from the octadecanoid signalling pathway several structurally non-related amino acid conjugates, like the bacterial phytotoxin coronatine[5], the synthetic indanoyl-isoleucine[6], or amino acid conjugates of linolenic acid (Lin-Ile) likewise induce volatile biosynthesis[3]. Minor changes in the amino acid moiety of the indanone conjugates result in different volatile profiles (sesqui- and diterpenoids), attributing to the amino acid substructure a specific role for the recognition and the selective induction[7]. In general, amino acid conjugates with fatty acids like linolenic acid or jasmonic acid need to be hydrolysed by phytogenic enzymes to gain biological activity, but the conjugates coronatine and indanoyl-isoleucine exhibit biological activity only as intact molecules[7].

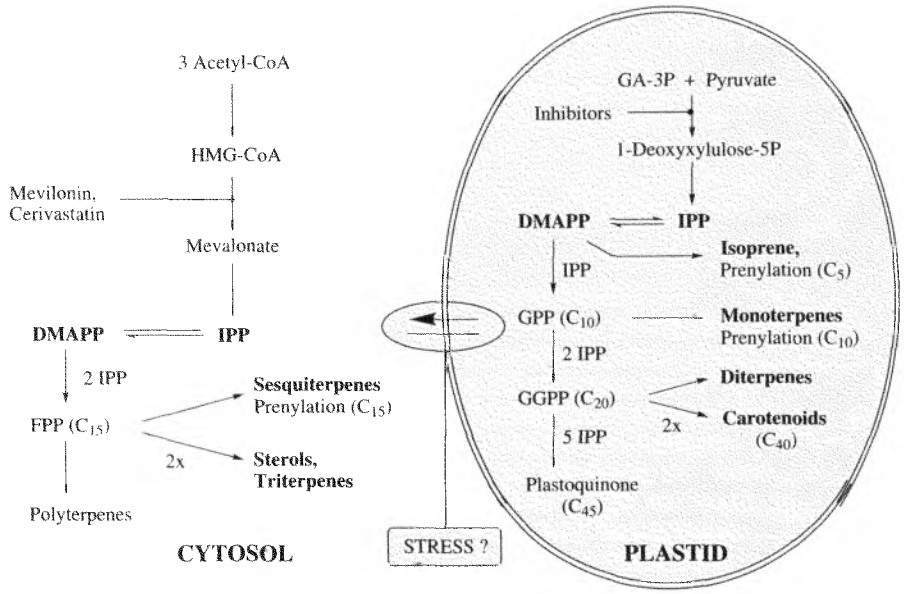

Fig. 3 Localisation of the mevalonate-dependent and the mevalonate-independent biosynthesis of terpenoids in cytosolic and plastidic compartments of the cell

(3) The 6-azido indanone (Fig. 2) was developed as a photolabile "elicitor" principally useful for a photoaffinity approach to study the late events of plant signalling (binding proteins, receptors)[8]. Details of JA/PDA-recognition and transduction of this signal into altered gene expresion are not known.

(4) The origin (mevalonate-dependent and mevalonate-independent biosyntehsis) of the induced terpenoids was studied by feeding labelled mevalonate or labelled deoxy-D-xylulose to herbivore-infested or JA-treated plants. Mass spectroscopic analysis of the emitted terpenoids revealed that mono- and diterpenoids are synthesised *de novo* along the novel desoxy-D-xylulose (DOX) pathway, while the biosynthesis of sesquiterpenes may be fuelled from both, the DOX- and the mevalonate pathway. Since also many sesquiterpenes were found to be highly labelled, these data support that DOX-derived precursors like IPP or GPP are shuttled from the plastid to the cytosol of the cell[9]. The extent of shuttling labelled precursors into the cytosol is especially high, if the cytosolic IPP-synthesis is blocked by inhibitors. This finding may be of importance for the maintaining the success of the plant defense in case of introduction of inhibitors along with the salivary secretion of herbivores. Similar findings concerning the efficiency of incorporation of labelled DOX into mono/di- and sesquiterpenoid volatiles have been made for flower volatiles.

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NEUROSTEROIDS: MEDICINAL CHEMISTRY OF STEROIDS AFFECTING GABA RECEPTOR FUNCTION

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Hans Selye showed in 1941 that pharmacological doses of progesterone caused general anesthesia in rats[1]. His discovery led to the development of intravenous anesthetic steroids by the pharmaceutical industry[2]. It is now established that the anesthetic action of these steroids is correlated with their enhancement of $GABA_A$ receptor-mediated neuronal inhibition[3].

Structure/activity studies have established the structural features that a steroid must contain for it to have anesthetic activity[2]. These structural features are present in the potent steroid anesthetics 3α5αP and 3α5βP (Figure 1). The 3α-hydroxy group, which appears to function as a hydrogen bond donor, is an absolute requirement. For optimal anesthetic activity, a group which can function as a hydrogen bond acceptor (*e.g.*, the acetyl group found in 3α5αP and 3α5βP or the cyano group found in 3α5αACN and 3α5βACN) is also required as a 17β-substituent.

The location of the binding site(s) of anesthetic steroids on $GABA_A$ receptors has (have) not been established. Moreover, there are no radiolabeled ligands for the putative steroid binding sites on $GABA_A$ receptors. Thus, it remains possible that steroid modulation of $GABA_A$ receptor function results from changes in receptor function caused by steroid-induced membrane perturbation rather than by the direct binding of these compounds to $GABA_A$ receptors. Studies of the membrane perturbing effects of anesthetic steroids indicate that the

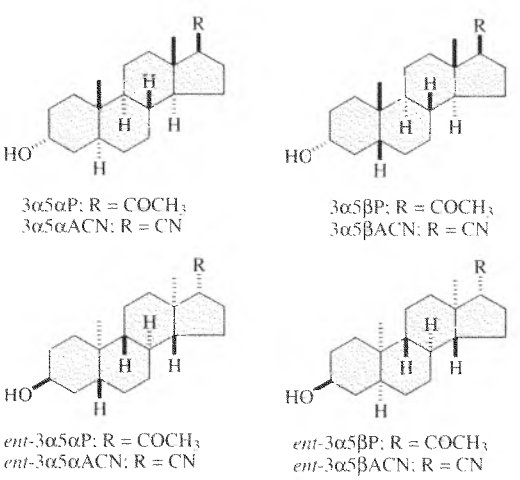

Fig. 1

structure/activity relationships that correlate steroid structure with $GABA_A$ receptor modulation also correlate with steroid-induced membrane perturbation[4].

To distinguish $GABA_A$ receptor modulation caused by the membrane perturbing effects of anesthetic steroids from modulation caused by steroid binding to the receptor protein, we

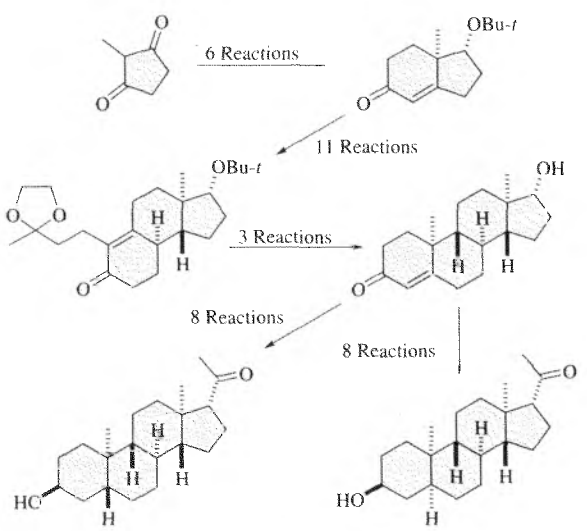

Fig. 2

have investigated the enantioselectivity of anesthetic steroid action[5]. No enantioselectivity would be expected for modulation of receptor function caused by anesthetic steroid-induced membrane perturbation. Conversely, enantioselectivity would be expected if the anesthetic steroids interact directly with the protein.

The non-naturally occurring enantiomers (ent-steroids) of four anesthetic steroids (Figure 1) have been prepared by total steroid synthesis[6,7]. The synthetic route used is briefly summarized in Figure 2. Four different biological assays have been used to evaluate the enantioselectivity of anesthetic steroid action. Enantiomer pairs were examined for their ability to: 1) non-competitively displace a radioligand bound at the picrotoxin binding site of $GABA_A$ receptors; 2) potentiate the actions of GABA-mediated chloride currents in cultured rat hippocampal neurons; 3) cause a loss of righting reflex in *Xenopus lavis* tadpoles and 4) cause a loss of righting reflex in mice. Except in the case of the 3α5βP, ent-3α5βP and 3a5βACN, ent-3α5βACN enantiomer pairs in the tadpole loss of righting reflex bioassay, where no significant enantioselectivity was found for their actions, the natural enantiomers were more potent anesthetics and $GABA_A$ receptor modulators than the unnatural enantiomers. The degree of enantioselectivity found for the 5α-reduced steroids was uniformly higher than that found for the 5β-reduced steroids. The results support the hypothesis that anesthetic steroids modulate $GABA_A$ receptor function by binding to the receptor.

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NON-ENZYMATIC CYCLIZATION OF ANALOGS OF OXIDOSQUALENE

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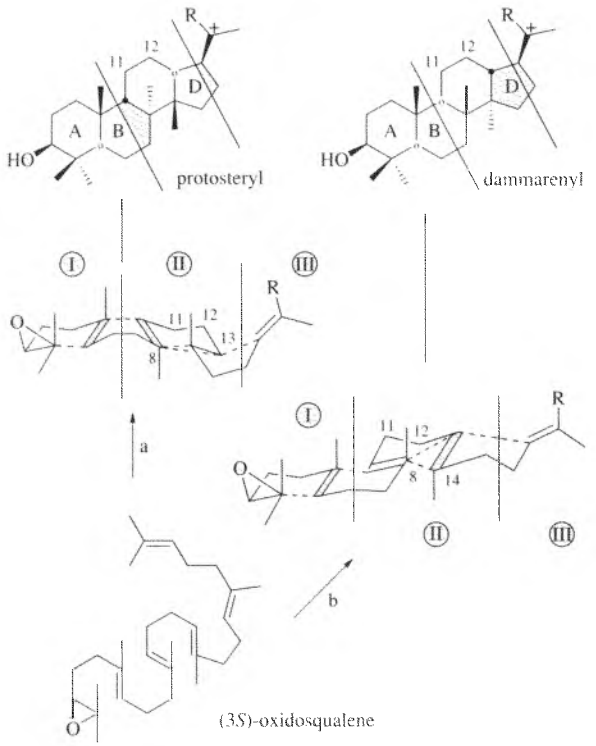
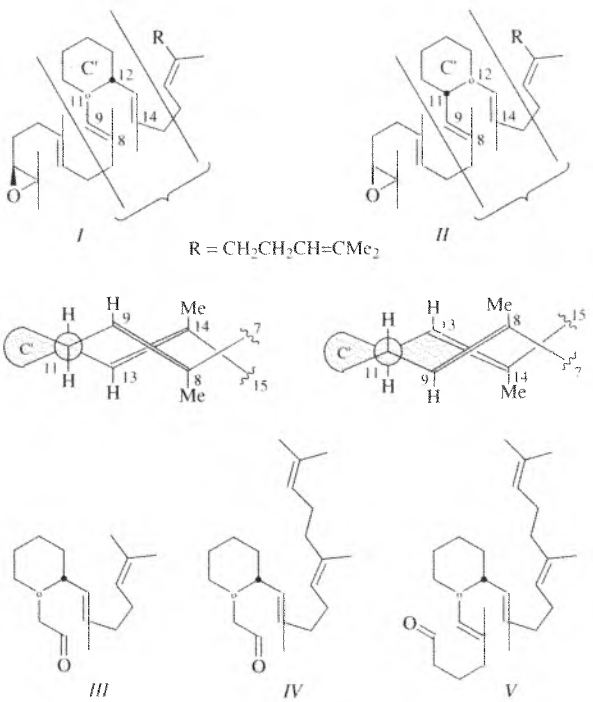
The polycyclization of (3*S*)-2,3-oxidosqualene to the tetracyclic protosteryl cation, one of the several steps involved in the biosynthesis of steroids, still remains a unique transformation. The resulting stereochemistry is in accord with a cyclization with the polyene folded in a prechair-preboat-prechair conformation (pathway a). The analogous cyclization leading to pentacyclic triterpenes via the dammarenyl cation would proceed through the corresponding prechair-prechair-prechair geometry (pathway b)[1]. Following the 1955 Stork–Eschenmoser postulate[2], systematic studies, in particular in the laboratories of Johnson[3] and of van Tamelen[4], have confirmed that cation-induced concerted polyene cyclizations do occur via antiparallel addition, hence with conservation of the π-stereochemistry, in a process whereby the resulting six-membered rings normally adopt chair conformations, and where the regioselectivity issue is invariably regulated by the stability of the resulting carbocations in compliance with Markovnikov's rule[5]. The quest of uncovering the exact role of the enzyme in the biogenetic cyclization has initiated decades of bioorganic studies in which the enzymatic cyclization of a variety of oxidosqualene analogs was investigated[1]. These have led to a detailed understanding of the substrate structural requirements and of the specificity and stereochemistry of the cyclization[6–8]. Whereas most aspects of the biocyclization have been successfully reproduced under nonenzymic conditions, the non-Markovnikov ring C closure remains excep-

tional; indeed, upon acid treatment of oxidosqualene there have been isolated, besides a bicyclic product with rearranged backbone (20–25% yield), two tricyclic products (25–30% yield) with a five-membered C-ring in accord with the formation of the thermodynamically favored tertiary cation[9].



The present study is part of a project in which we wish to study the possibility that the full course of the oxidosqualene cyclization would be determined by one specific localized torsion at bond 11–12 in the acyclic precursor polyene[10]. The following observations are at the basis of this hypothesis. Of the three regions I–III that one may distinguish within the cyclization process, the central region II is the most critical: (i) its orientation relative to the (*S*)-epoxide moiety determines, at least in a concerted pathway, if the protosteryl or dammarenyl cation is formed; (ii) it incorporates the chemically unchallenged anti-Markovnikov closure of ring C; and (iii) it may also be involved in the final construction of the side chain, in particular the stereospecific obtention of C-20. The enantiomeric relation of this particular region in both series and the presence of a pseudo-C_2 axis through bond 11–12 within region II suggest that the sense of chirality of the torsion at 11–12 could eventually determine the outcome of the process. In addition, the magnitude of the torsion could be responsible for induction of anti-Markovnikov C-ring closure. In this context the study of the ring closure of epoxides such as *I* and *II* should allow for eventual confirmation. The role of the six-membered C′-ring at 11–12 consists of enforcing the relative orientation of the two polyene chains in one or the other chiral sense as illustrated by the Newman projections, hence determining the cyclization pathway that is followed. Moreover, due to the presence of this extraneous ring, ring C upon concerted cyclization becomes connected to two other rings (*i.e.*, the B- and C′-rings) *via trans*-fusions at 11–12 and at 8–9. The resulting strain is expected to be better accommodated by a six-membered than a smaller five-membered ring size, hence possibly favoring ring closure toward the anti-Markovnikov product. Finally, due to the presence of the C′-ring the proximity of C-8 and C-14 is enforced. This should ease the entropic burden inherent in a polycyclization and help favor a concerted pathway beyond the second cyclization.



In this context we will describe the results of the Lewis acid catalyzed cyclizations of polyene aldehydes with increasing structural complexity, *i.e. III*, *IV* and *V*, designed so as to enforce ring closure to the "natural" CD-ring system.

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10. The numbering used is consistent with the usual steroid numbering.

IDENTIFICATION AND SYNTHESIS OF NEW TERPENOIDS FROM INSECTS

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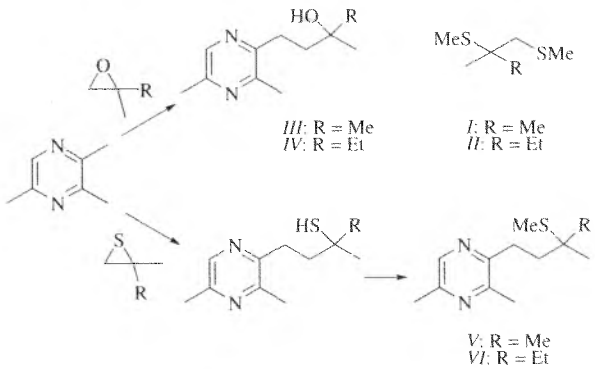
Insect released isoprenoids exhibit a multitude of biological and physical functions. In intraspecific communication they may play a role as chemical signals to attract and/or alarm conspecifics. In interspecific competition they may be used during aggression or defense. They may also act as "solvents" for other compounds like poisonous alkaloids which they carry through the cuticle of prey[1].

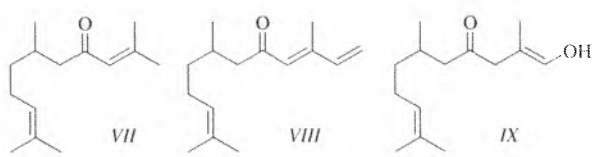
Some new structures from various insects will be presented in four small sections. Their structure elucidation and synthesis as well as their possible biosynthesis will be discussed. Results have been obtained in close cooperation with biologists and chemists.

Longhorn beetles[2]. Compounds *I–VI* were identified in the metathoracic gland of the longhorn beetle, *Pyrrhidium sanguineum*.

The compounds may play a role in interspecific competition, specially in defense against ants. Though the pyrazines *III* and *V* show a typical isoprenoid side chain, it seems to be more likely that the biosynthesis of all compounds involves amino acids, especially valine and/or leucine/isoleucine, rather than mevalonate/homomevalonate.



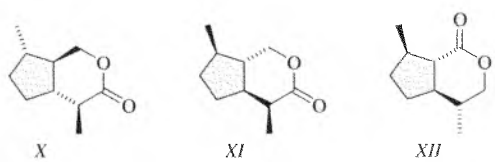
Cuckoo bees[3]. The mandibular gland secretions of many females of cuckoo bees, *Nomada* spp., are made up by species specific blends of sesquiterpene derivatives, dissolved in mixtures of long chain hydrocarbons[4]. Major terpenes are represented by compounds *VII*, *VIII*, and *IX* as well as its esters with common fatty acids. Males frequently contain esters of farnesol with hexanoic or octanoic acid.



The compounds seem to play a role in intra- and interspecific competition.

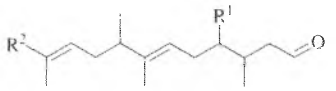
Hyperparasitoids[5]. Volatile compounds which play a role in the tetratrophic system oats, *Avena sativa* - aphid, *Sitobion avenae* – parasitoid, *Aphidius uzbekistanicus* – hyperparasitoid, *Alloxysta victrix*, were investigated. Several monoterpenoids were found in the cephalic secretions of *Alloxysta victrix*. Particularly interesting are the *trans*-fused iridoids *X–XII*.

All stereoisomers of *X* and *XII* could be prepared starting from pure enantiomers of limonene.



Ants[6]. The Dufour glands of worker ants of *Tetramorium caespitum* and related species are filled with straight chain hydrocarbons and a series of homologous sesquiterpenes which have been termed "tetramorines" (ref.[7]).

Coupled gas chromatography/mass spectrometry and synthetic approaches proved the compounds to be the aldehydes *XIII–XV* (tetramorins 1–3). The stereochemistry of the compounds is unknown.



XIII: R^1= H R^2= Me
XIV: R^1= Me R^2= Me
XV: R^1= Me R^2= Et

The biosynthesis may involve 1–3 homomevalonate units. The biological function of the compounds is unknown.

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THE METABOLIC TRIAD OF PHYTOESTROGENS. THE IDENTIFICATION AND SYNTHESIS OF NOVEL ISOFLAVONOID METABOLITES OF DAIDZEIN AND/OR GLYCITEIN

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Isoprenoids comprise a family of more than 23 000 natural products, among them the precursors of cholesterol and taxol. The renewed interest in the medicinal value of natural plant products have redefined the structural significance of isoprenoids. Steroids are derived from isoprenoid units. Unlike isoprenoids, flavonoids/isoflavonoids are today accepted to be derived by a combination of a shikimate-derived C_6-C_3 group with a six-carbon polyketide chain, formed by the union of one acetate and two malonate residues. Subsequent ring closure leads to chalcones, the precursors of the whole gamut of flavonoid/isoflavonoid compounds which together with lignans form the group of plant derived estrogenic diphenols referred to as "phytoestrogens". It is the structural resemblance and functional significance of phytoestrogens with endogenous steroid estrogens that has made isoflavonoid phytoestrogens one of the most growing fields of research in recent years.

Phytoestrogens are found in whole soybeans, soybean, legumes, fruits as well as whole grain cereals, seeds and nuts. On consumption by humans, these undergo metabolic changes. The resulting metabolites though structurally similar to the phytoestrogen precursors, these are no longer plant substances but products of gut microbial transformations with weak estrogenic and antioxidative activity. As such phytoestrogen metabolism in humans is an interaction between dietary plant precursors, microbial transformations and endogenous hormonal changes through absorption of precursors and metabolites in human cells.

ISOFLAVAN **ANGOLENSIN**

Molecular structures of isoflavan and angolensin numbered accoording to *Chem. Abstracts*.

Phytoestrogens have now been shown to influence not only sex hormone metabolism and biological activity but also intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, differentiation and angiogenesis. Countries with high soy intakes have lower risks of cancers, which have been attributed to the high phytoestrogen content in the diet[1]. Animal studies provide interesting data suggesting that early exposure to isflavonoid phytoestrogens modifies biological effect[2].

In spite of the beneficial effects of phytoestrogens the mode of metabolism for the two major phytoestrogens found in soy namely daidzein and genistein was proposed only recently with the identification of a number of new metabolites in the urine of human volunteers consuming a diet enriched with soy[3]. More recently the identification of two new metabolites, 6,7,4′-trihydroxyisoflavanone and 5′-hydroxy-*O*-demethylangolensin [1-(2′,4′,5′-trihydroxyphenyl)-2-(4″-hydroxyphenyl)-propan-1-one], in the urine of human volunteers consuming a diet rich in phytoestrogens, provide evidence to support an alternative pathway of metabolism for daidzein and/or glycitein. The metabolic triad of phytoestrogens which exists between plants, microbes and humans will be discussed here together with the new data on the identification and synthesis of all metabolites identified so far emphasising the biochemistry and chemistry of phytoestrogen precursors and metabolites alike.

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STEROID, A STILL FASCINATING APPROACH TO NOVEL STRUCTURES: STEROKLASTANES AND STEROID TEMPLATE ASSOCIATED PEPTIDES

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The golden age of steroids appears today to be just a historical event remembered only by a few contemporaries. In those days, however, this vast area of natural product chemistry dominated the scientific activity of numerous academic and industrial chemical and biochemical research units, including Prof. Šorm's Institute of Organic Chemistry and Biochemistry in Prague, and stimulated the initiation of a great number of symposia, like today's Conference on Isoprenoids.

The impressive progress of biological sciences of the last two decades had, nevertheless, also a strong impact on chemistry, which is generally considered a "ripe" science. In the area of steroids various receptors which are essential for their biological activity have recently been characterized and the problem of molecular recognition is becoming even more relevant.

Among various possibilities for the synthesis of new steroids interacting more selectively with the envisaged macromolecules, there are two approaches which will be discussed in more detail.

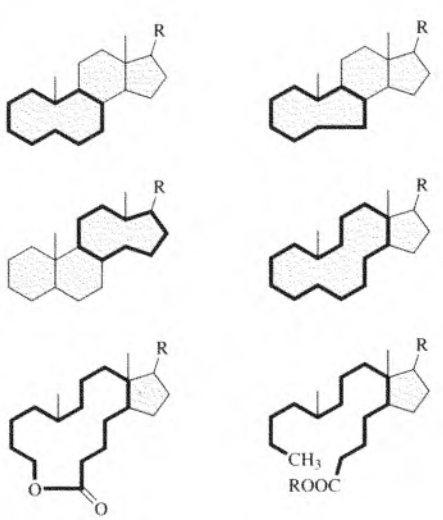

Fig. 1

The first (classical) access which has been followed extensively by colleagues at the University of Belgrade (groups of Profs. M. Lj. Mihailović and Lj. B. Lorenc) consists in the application of fragmentation reactions of *O*-radicals generated from corresponding hydroxy groups using lead tetraacetate and hypoiodite reactions developed in Basel, Zürich, and Belgrade. Starting from various intact cholestane and /or androstane derivatives, steroid analogues (steroklastanes) containing a medium-sized ring, macrolides or even prostanoid-type compounds (Fig. 1) are available.

A second approach is based on the general concept of prof. Mutter's TASP (**T**emplate **A**ssembled **S**ynthetic **P**roteins). We have tested the idea of fixing specific desamino analogues of amino acids, considered essential for the binding of a peptidic/proteinic substrate to the corresponding receptor or antibody, on a semi- rigid template (Template Assembled Surface Mimetics) (Fig. 2). The mimicking of interactions with one of the angiotensin II receptors serves as an example.

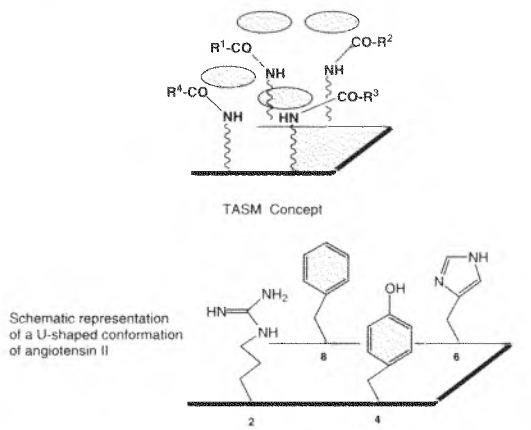

Fig. 2

A positive outcome of these experiments prompted us to use the framework of steroids as a rigid template which would allow us to forsee the spatial arrangement of the attached amino acids or peptides in adequate surface mimetics. At the same time the new project called STAP (**S**teroid **T**emplate **A**ssociated **P**eptides) should combine two important areas of natural product chemistry.

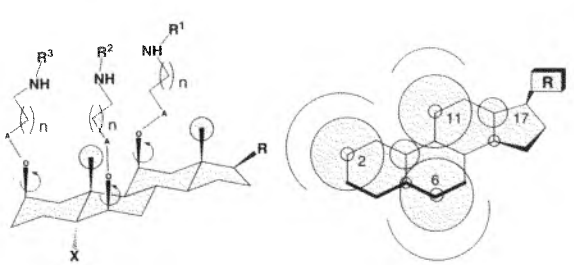

Fig. 3

A first variant of the above idea is schematically depicted in Fig. 3 wherein the amino acid residues are attached via appropriate spacers to the axial positions 11β and 6β or 2β. However, the experimentally followed route which led to the desired intermediates, proved to be unattractive and was not pursued further.

Another approach to STAP molecules which promises versatile applications (Fig. 4), would involve the introduction

of different peptidic chains into positions 18 and 19. In addition to the possibility to form a bridge between the two chains, additional interactions with substituents at $C_{(3)}$ and/or $C_{(17)}$ would be possible. In this way different parts of a protein surface could be mimicked, and partially rigidized loops and sheets matched.

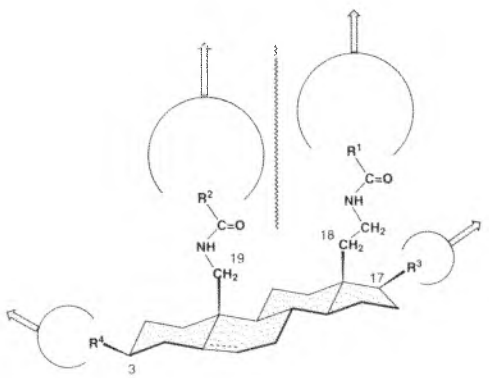

Fig. 4

Again, two different intramolecular free radical reactions which we have developed several years ago - the "Hypoiodite Reaction" and the "Oxidative Cyanohydrin-Cyanoketone Rearrangement"- proved to be particularly suitable for the synthesis of the envisaged STAP derivatives. Applying these procedures to 18- and 19-unsubstituted pregnanes helped to generate the first steroids containing a formal dipeptide sequence attached to these two positions.

NEUROSTEROIDS, DEFINITION, BIOCHEMISTRY, PHYSIOLOGICAL ASPECTS

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Definition: the term neurosteroids was proposed in 1981. It applies to the steroids, the accumulation of which in the central and peripheral nervous systems occurs independently, at least in part, of supply by the steroidogenic endocrine glands, and which can be synthesized *de novo* in the nervous system from sterol precursors[1]. All intermediary compounds can be assayed and/or demonstrated to be formed *in situ*. On the contrary, those steroids which are formed exclusively from blood borne precursors, as for example estrogens (estrone and estradiol) which derive by aromatization from blood borne androstenedione and testosterone, will not be qualified as neurosteroids.

There is a tendency in the scientific literature to quote as "neurosteroids" all neuroactive steroids, including synthetic, non-natural molecules. This is unfortunate. The definition *stricto sensu* of neurosteroids applies to PREG, DHEA, and their sulfate and fatty acid esters, to PROG and to its 5α-reduced metabolites (5α-DH PROG, 3α, 5α-TH PROG, and 3β, 5α-TH PROG).

Biochemistry. It is not our purpose to provide a detailed description of the biosynthetic and metabolic enzymes. Our current view of neurosteroid biosynthesis and metabolism in the rat brain is summarized on Figure 1.

Solid evidence has been provided for the biosynthesis of PREG in the nervous system, whereas the pathway(s) by which DHEA biosynthesis occurs in the brain remain(s) controversial. However, Prasad *et al.* have suggested an hypothetical biochemical pathway from cholesterol to PREG and DHEA: the fragmentation of *in situ* - formed tertiary hydroperoxides ("hydroperoxide pathway")[2].

Physiological aspects. There are still little functional correlates of neurosteroids suggesting their physiological implication in the functioning of the nervous system. Here are reviewed three such examples. Two of them deal with the implication of PREG S in a particular type of aggressive behaviour and in memory performance, the third one relates to the role of progesterone in peripheral nerve regeneration.

Pregnenolone sulfate and the aggressive behavior of mice against lactating female intruders. This peculiar model of aggressiveness is influenced by the genotype of the mice and by their sex: females are more aggressive than males. Castration of males triggers a marked increase of aggressiveness, and their treatment with testosterone or estradiol counteracts the effect of castration. DHEA also inhibits the aggressiveness of castrated male mice. To completely eliminate the possibility of an androgenic action of DHEA, the effect of its analog 3β-methyl-androst-5-en-17-one (CH_3-DHEA) was investigated. This molecule cannot be metabolized into sex steroids and is not demonstrably estrogenic or androgenic. Nevertheless it inhibited the aggressive behaviour of castrated mice dose-relatedly, at least as efficiently as DHEA itself.

Both DHEA and CH_3-DHEA (280 nmol/day for 2 weeks) produced a marked and significant decrease of PREG S concentrations (more than two fold) in the brain of treated castrated mice. Neither testosterone nor estradiol mimicked this effect of DHEA and CH_3-DHEA. We have speculated that the decrease of PREG S levels might increase the calming GABAergic tone, which has repeadly been implicated in the control of aggressiveness, and possibly may decrease also the activity of "excitatory" NMDA receptors. The time-course of PREG S decrease in brain following DHEA administration supports this conclusion. Indeed, the castrated mice had to be treated for 2 weeks with DHEA before getting a clear-cut, significant inhibitory effect on aggressiveness. Accordingly, the decrease of PREG S in brain was gradual, and became significant only 15 days after the onset of treatment. The influence of DHEA treatment was also investigated in females, together with its eventual modulation by testosterone injected to the newborn. Indeed, the females which had been androgenized at birth, then treated as adults with DHEA, were much less aggressive towards lactating intruders than normal females. In accordance with this observation, the decrease in the concentration of PREG S produced by DHEA, already significant in the group of spayed mice treated with DHEA, was signifi-

cantly larger in the subgroup of neonatally androgenized females treated with DHEA. The mechanisms by which DHEA or CH_3-DHEA decrease PREG S concentrations in brain are unknown. An inhibition of sulfotransferase activity might be involved.

Pregnenolone sulfate and memory performance of aged rats. Cognitive abilities exhibit a natural decline with age although with considerable interindividual differences, which have been exploited in the search for relationships between the performance of each ≈2 years old rat and PREG content in several areas of its brain, particularly in the hippocampus. The spatial memory performances of aged rats were investigated in two different spatial memory tasks, the Morris water maze and the Y-maze. Performances in both tests were correlated and, accompanied by appropriate controls, were considered to evaluate genuine memory function.

The fundamental observation was that individual hippocampal PREG S levels and distance to reach the platform in the water maze were linked by a significant negative correlation, *i.e.* these rats with no or minimal memory deficits had the highest PREG S levels, whereas no relationship was found with PREG S content in other brain areas (amygdala, prefrontal cortex, parietal cortex, striatum). As a confirmation for the role of PREG S, the memory deficit of cognitively impaired aged rats was transiently corrected after either intraperitoneal or bilateral intrahippocampal injection of PREG S.

The hippocampal content of PREG S seems to play a physiological role in preserving and/or enhancing cognitive abilities in old animals, possibly via an interaction with central cholinergic sytems. Neurosteroids should be further studied in the context of prevention and/or treatment of age related

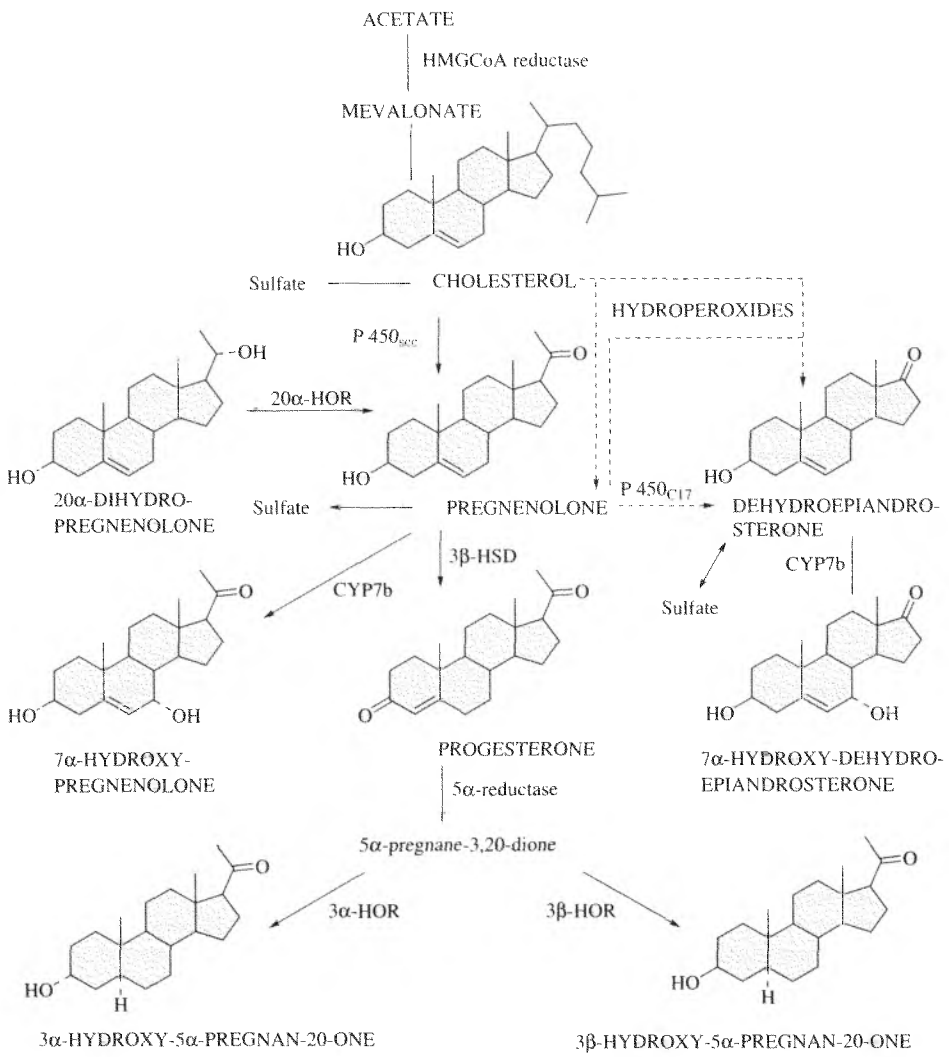

Fig. 1. Neurosteroid biosynthesis and metabolism in the rat brain. Dotted arrows indicate metabolic conversions not yet formally demonstrated

memory disorders. For that purpose, methods should be developed to produce a sustained correction of PREG S deficits in the hippocampus of aged rodents.

Locally synthesized progesterone promotes myelination in peripheral nerves. Peripheral nerves are particularly well suited to explore the trophic functions of neurosteroids because of their relatively simple structure, their great plasticity and their remarkable regenerative capacity. In response to a cryolesion, axons and their myelin sheaths distal to the lesioned site rapidly degenerate by a process known as Wallerian degeneration, but Schwann cells survive and proliferate. Such lesion leaves basal lamina tubes of the nerve fibers intact and provides an appropriate environment for a rapid regeneration of the damaged axons, which are then remyelinated by Schwann cells and eventually make new functional neuromuscular connections. In the intact and in the regenerating nerve, concentrations of PROG are about 6-fold larger than in plasma (plasma: 1.3 ± 0.1 ng/ml; nerve: 8.5 ± 0.9 ng/g). Blocking either its local synthesis or action by repeated applications of either trilostane (an inhibitor of 3β-HSD) or RU486 (an antiprogestin) to the regenerating nerve, inhibits the formation of new myelin sheaths after cryolesion. Conversely, repeated local administration of a high dose of PROG or its direct precursor PREG (100 µg), accelerates the process of remyelination. In these experiments, the width of myelin sheaths was analyzed by electron microscopy on cross-sections two weeks after lesioning.

Schwann cells not only have the capacity to synthesize PROG from PREG, they also express an intracellular PROG receptor. In addition, PROG activates the expression of genes encoding the peripheral myelin proteins P0 and PMP-22. As dosage of P0 and PMP-22 gene expression plays an important role in peripheral neuropathies, their reduced or increased expression leading to demyelination and eventually axonal degeneration (132), PROG may play a significant role in the pathophysiology of these diseases and may provide opportunities for their treatment. Preliminary findings strongly suggest that PROG may also promote myelination in the CNS. The mRNAs for cytochrome P450scc and 3β-HSD are induced during the differentiation of oligodendrocyte progenitors. Like Schwann cells, oligodendrocytes express intracellular PROG receptors and, in cultures of glial cells prepared from newborn rat brain, the number of oligodendrocytes expressing the myelin basic protein is increased by PROG.

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ISOPRENOID BIOSYNTHESIS IN BACTERIA AND PLANTS; THE ELUCIDATION OF THE MEVALONATE-INDEPENDENT METHYL ERYTHRITOL 4-PHOSPHATE PATHWAY.

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Feeding experiments performed with liver and yeast for instance using precursors labeled with radioactive or stable isotopes showed the key role of mevalonate (*IV*) for the biosynthesis of cholesterol in liver or ergosterol in yeast. Confirmation of this precursor role of mevalonate was also obtained for the biosynthesis of other isoprenoids, including isoprenoids from plants and bacteria. Mevalonate synthesized from acetyl coenzyme A (*I*) *via* acetoacetyl coenzyme A (*II*) and hydroxymethylglutaryl coenzyme A (*III*) (Fig. 1) was consequently unanimously accepted as the precursor of all isoprenoids in all living organisms, despite some contradic-

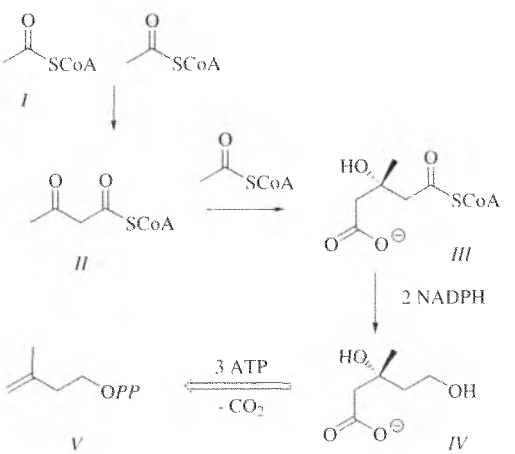

Fig. 1. Mevalonate pathway for isoprenoid biosynthesis

tory results concerning the biosynthesis of some isoprenoids from plants or from bacteria. Incorporation of ^{13}C labeled acetate or glucose into bacterial isoprenoids such as triterpenoids of the hopane series, prenyl chains of ubiquinone and menaquinone or carotenoids allowed the detection and partial elucidation of a novel mevalonate-independent route to isopentenyl diphosphate (*V*) (IPP), the universal precursor for isoprenoids (Fig. 2)[1-4].

Glyceraldehyde 3-phosphate (*VI*) (GAP) and pyruvate (*VII*) are the first precursors[3,4]. Condensation of (hydroxyethyl)thiamin, resulting from pyruvate decarboxylation, on the carbonyl group of GAP yields 1-deoxy-D-xylulose 5-phosphate (*VIII*) (DXP). Incorporation of deuterium labeled free deoxyxylulose into the ubiquinone and menaquinone of *E. coli* pointed out the role of isoprenoid precursor of this pentulose[3], which is also the precursor of

pyridoxol phosphate and of the thiazole moiety of thiamine diphosphate. The branched carbon skeleton of isoprenic units results from the rearrangement of the straight-chain DXP (refs[4,6]). Identity of the C_5 skeletons of DXP and IPP was demonstrated by incorporation of deoxyxylulose isotopomers with multiple ^{13}C labeling[7,8].

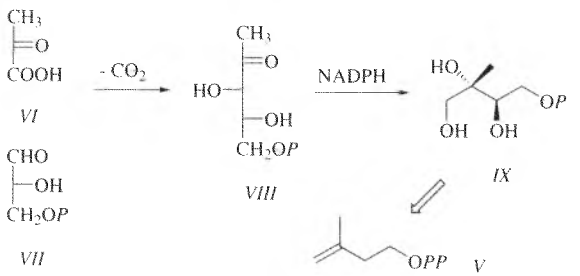

Fig. 2. Methylerythritol 4-phosphate pathway for isoprenoid biosynthesis

2-*C*-Methyl-D-erythritol cyclodiphosphate, the free tetrol or the corresponding lactone were found in several bacteria and higher plants. These tetrol derivatives fitted well in our hypothetical biogenetic scheme[4]. Feeding of ^{13}C labeled glucose to *Corynebacterium ammoniagenes* showed that the prenyl chain from dihydromenaquinone and 2-*C*-methyl-D-erythritol resulted both from the mevalonate-independent route[9]. Furthermore, the deuterium labeled D-enantiomer was incorporated into the prenyl chains of ubiquinone and menaquinone of *Escherichia coli*, indicating a possible precursor/product relationship. No other intermediates have been presently identified[10].

Two enzymes of this metabolic route were identified. The gene of the DXP synthase was cloned in *Escherichia coli*[11,12] and peppermint[13], and that of the DXP reducto-isomerase catalyzing the concomitant rearrangement of DXP and the reduction of the resulting aldehyde into 2-*C*-methyl-D-erythritol 4-phosphate (*IV*) (MEP) in *Escherichia coli*[6]. MEP with its branched C_5 carbon skeleton can be considered as an hemiterpene and might represent the first committed intermediate of this metabolic route[14].

The GAP/pyruvate route is found in many eubacteria, including pathogens and opportunistic pathogens, and in unicellular algae (Chlorophytes, Rhodophytes, Chrysophytes). In higher plants, this bacterial route is involved in the biosynthesis of all essential chloroplast isoprenoids (*e.g.* phytol, carotenoids, plastoquinone)[15] as well as in the formation of other plastid related isoprenoids of more restricted distribution such as isoprene, monoterpenes and diterpenes[14,16].

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BRASSINOSTEROID METABOLISM

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In 1979 Grove *et al.* published the structural elucidation of brassinolide, (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-6a-oxa-5α-cholestan-6-one (Fig. 1, *I*), being the first and hitherto most active member of a unique family of steroidal plant growth regulators[1].

An amount of only 4 mg of crystals of *I* were obtained from 40 kg rape pollen (*Brassica napus*), indicating occur-

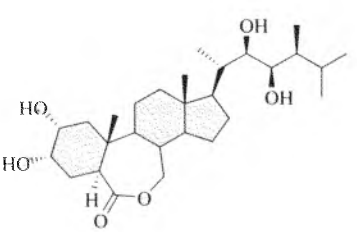

Fig. 1. Structure of brassinolide (*I*)

rence in very low concentrations. The high activity at these low concentrations on cell elongation, cell division and many other physiological processes stimulated intense research activities in many laboratories. Remarkably, the activity of brassinolide was shown to be different from that of classical phytohormones and other plant growth regulators. Many studies confirmed the ubiquitous presence of brassinolide-related compounds, collectively named brassinosteroids, in the plant kingdom. Further research efforts were directed towards chemical synthesis, biochemistry, biological mode of action, and practical application in agriculture. Despite evidence provided by extended biological studies, the status of brassinosteroids as a group of phytohormones was not generally accepted until recently. However, investigations involving a number of brassinosteroid deficient and perceptive mutants of *Arabidopsis thaliana*, *Lycopersicon esculentum* and *Pisum sativum* have established the essential role of brassinosteroids in plant growth and development (for review, see[2]).

The biosynthesis of brassinosteroids was studied by Japanese research groups, feeding tritiated or deuterated precursors to cell cultures of *Catharanthus roseus* (for review, see[3]). These studies confirmed campesterol (*II*) as precursor of brassinolide (*I*). The multistep biosynthetic pathway involves 5α-reduction of *II* to form the *trans*-fused A/B ring system followed by a sequence of oxidation steps and, in between, inversion of configuration at C-3. Two alternative routes comprising identical numbers of biosynthetic steps were found, being different only in oxidation at C-6 occurring early between campestanol (*III*) and 6-oxocampestanol (*IV*) or later converting 6-deoxocastasterone (*V*) to castasterone (*VI*) (Fig. 2).

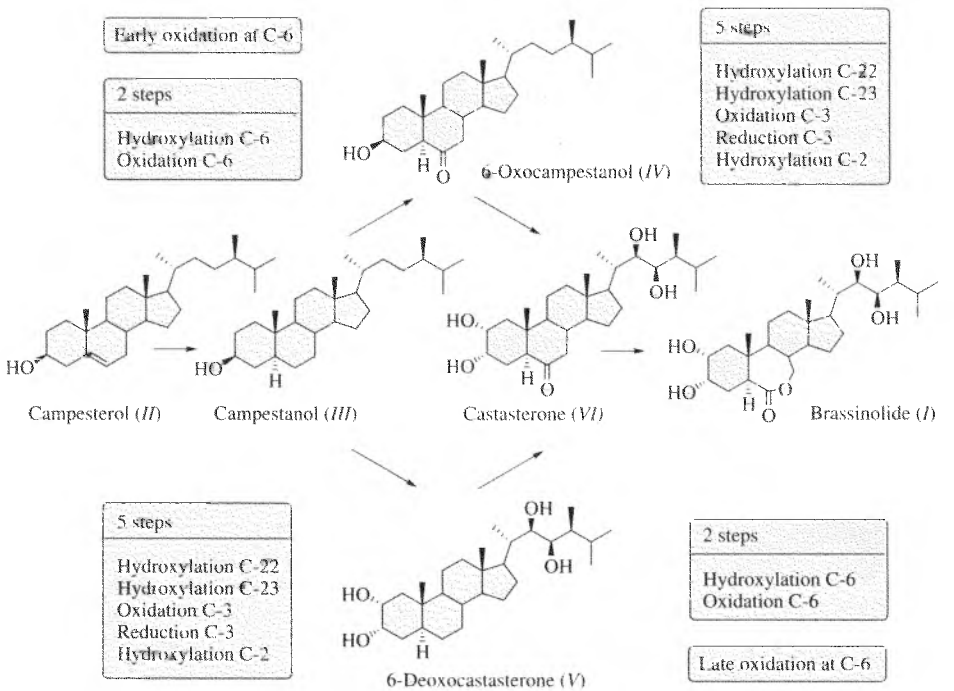

Fig. 2. Alternative early and late C-6 oxidation pathways of brassinolide biosynthesis (for review, see ref.[3])

Further variations of the functionalization sequence can be supposed. Moreover, it seems most likely that phytosterols having different side chains structures are converted to brassinosteroids in parallel pathways without altering the carbon scaffold. A number of steps in the biosynthesis of 24*R*

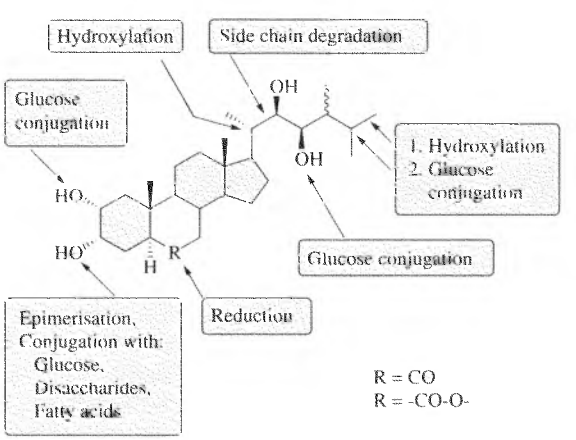

Fig. 3. Reactions observed in brassinosteroid metabolism

methyl brassinosteroids, namely epimerization of 24-epiteasterone (*VII*) at C-3 *via* 3-dehydro-24-epiteasterone (*VIII*) to afford 24-epityphasterol (*IX*), and the Baeyer–Villiger reaction converting 24-epicastasterone to 24-epibrassinolide, were demonstrated *in vitro* using enzyme preparations of *Lycopersicon esculentum* cell cultures[4]. Combination of feeding experiments and molecular analysis of brassinosteroid biosynthetic mutants, whose dwarf phenotype can be rescued to wildtype phenotype by exogenous brassinosteroid treatment, revealed the sites of lesions in the pathway. Cloning, heterologous expression, and sequence analyses indicated that at least two hydroxylation steps involved in brassinolide biosynthesis are catalysed by P450 monooxygenase type enzymes. Further mutants are deficient in enzymes catalyzing early steps of brassinosteroid biosynthesis, namely before campesterol (*II*) or between *II* and campestanol (*III*) (for review, see[5]).

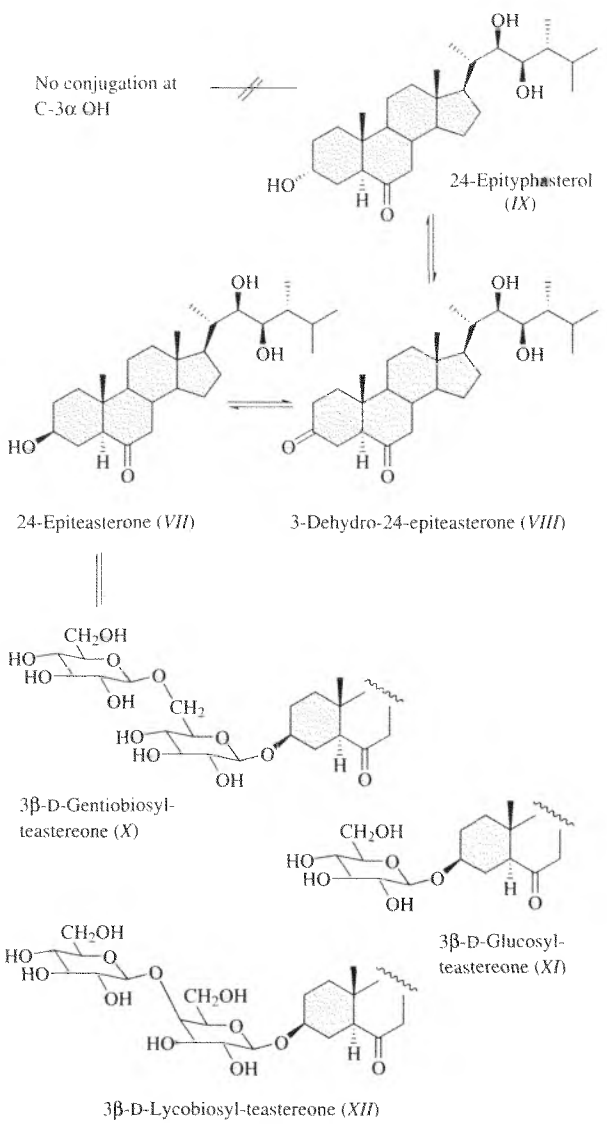

Fig. 4. Reversible epimerization and selective glycosidation of 24-epiteasterone in tomato cell cultures

Detailed studies on metabolism and conjugation of brassinosteroids were carried out using cell cultures of *Lycopersicon esculentum* and *Ornithopus sativus* (for review, see[6]). About 30 metabolites have been isolated and identified upon administration of 24-epicastasterone, 24-epibrassinolide, and 24-epiteasterone (*VII*), mostly being novel metabolites formed by hydroxylation, epimerization, side chain cleavage, reduction, and conjugation (Fig. 3). A variety of novel pentahydroxylated metabolites carrying additional hydroxyl groups at C-20, C-25, and C-26, respectively, was found. While hydroxylation at C-20 is followed by side chain removal yielding pregnane type compounds, new hydroxyl groups at C-25 and C-26 rapidly undergo glycosidation. For stereochemical reasons, conjugation at C-3 requires 3β-configuration. This was demonstrated, for example, by smooth glycosidation and acylation of 24-epiteasterone (*VII*) and other C-3β compounds. In contrast, C-3α brassinosteroids did not undergo conjugation. Selective reversible conjugation, together with reversible epimerization, may provide the opportunity to regulate the cellular level of hormonal active brassinosteroids (Fig. 4).

Biosynthetic and metabolic studies on brassinosteroids are essential to better understanding the molecular mode of action of brassinosteroids. Chemical, biochemical, physiological and molecular investigations are expected to provide information on the precise role of these of phytohormones as well as on plant growth and development in general.

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STEREOCONTROLLED SYNTHESIS OF ISOPRENOID INSECT PHEROMONES AND RELATED TARGETS

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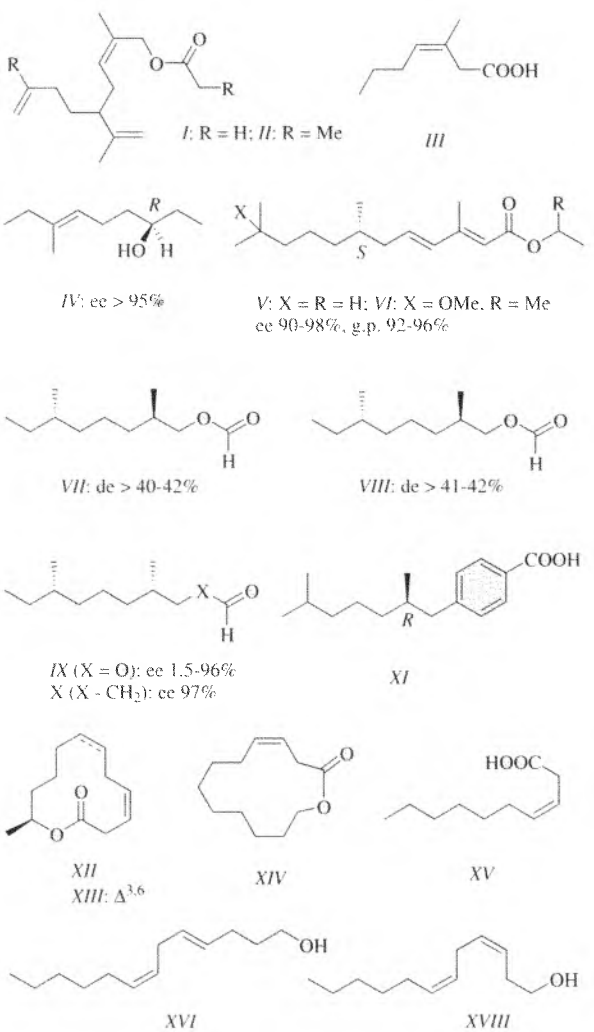
Three operationally simple protocols for stereocontrolled synthesis of insect behaviour- and growth-regulating substances of isoprenoid and acetogenin origin have been developed since 1994 in our laboratory:

(*1*) A sequence that combines the Horner–Emmons olefination of aldehydes by bifunctional allylic phosphonates with Frankel's 1,4-*cis*-hydrogenation of the resulting alkyl 2,4-alkadienoates to give optionally *Z*- or *E*-configured trisubstituted olefins depending on the structure of the diene. Some new modifications of this protocol were also elaborated, *e.g.*, the synthesis of "skipped" *Z,Z*-diolefins.



(*2*) An alternative route to the required 2,4-alkadienoate substrates that employs the Claisen–Johnson rearrangement followed by the introduction of an arylseleno group in the α-position of the resulting ester and its subsequent oxidative elimination (a large part of this work was done in co-operation with Dr. Lars Engman at the University of Uppsala, Sweden).

(*3*) Stereodivergent synthesis of all stereoisomers of a given chiral biomolecule that employs the PPL-mediated kinetic resolution of racemic precursors as the key step. A new modification of this protocol consists in enhancing the enantioselectivity of resolution of an aromatic substrate by temporarily converting the latter into a (η^6-arene)chromium tricarbonyl complex.

Using these protocols some pheromones (*I–IV, X, XII–XVII*), pheromone mimics (*VII, VIII*), JH analogues (*V, VI*), and a hypolipidaemic agent (*XI*) were obtained.

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INSECT INDUCED PLANT BIOSYNTHESIS OF VOLATILE ISOPRENOIDS

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Feeding damage by insect herbivores induces defensive chemical reactions by plants, one of which is an increase in

the release of volatile compounds. Natural enemies of the herbivores use these volatile semiochemicals to locate their hosts or prey. Although some volatile compounds are released from storage in plants immediately whenever damage to cells or glands occurs, others are synthesized *de novo* and released only during the light period. This often results in a delay between feeding damage and release of volatiles. Plants release the induced compounds from undamaged as well as damaged leaves. Thus, damage to only a few leaves results in a systemic response and release of volatiles by the entire plant. This herbivore induced *de novo* biosynthesis of volatile plant metabolites, derived from several different biochemical pathways (Figure 1), is triggered by interaction of elicitors in the oral secretions of insect herbivores with damaged plant tissues. Elicitors in the oral secretions of beet armyworm caterpillars have been identified as *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin) and the analogous *N*-linolenoyl-L-glutamine. Other analogs are present but are not active in inducing corn seedlings to emit volatiles. Labeling studies have shown that volicitin is produced in the beet armyworm from linolenic acid obtained from the plant on which the caterpillar feeds. Considerable evidence now exists to indicate that plants respond differently to individual herbivore species at least in part due to the composition of insect elicitors that interact with damaged plant tissues. Specialist parasitoids can differentiate the volatile blends released due to damage by hosts from those resulting from non-host damage as well as from mechanical damage, thereby facilitating host location for the parasitoid.

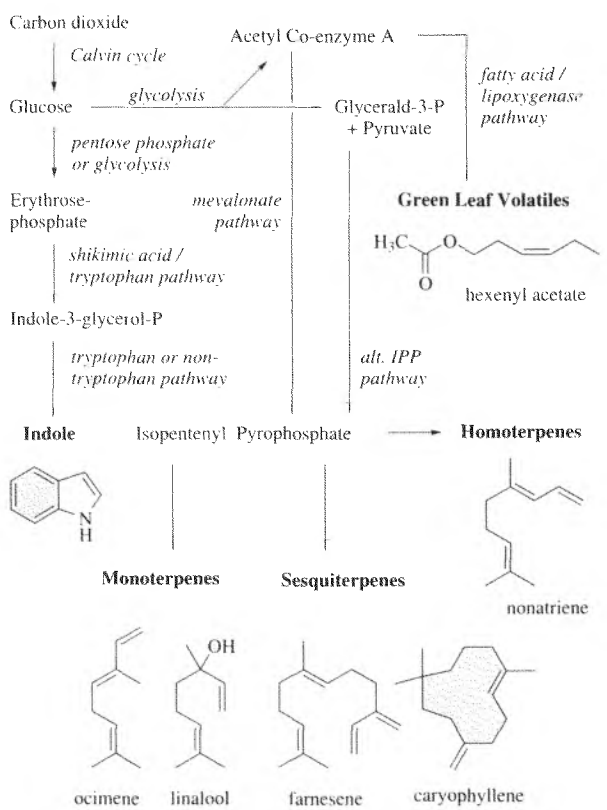

Fig. 1. Biosynthetic pathways leading to five distinct classes of plant volatiles; compound types are listed in bold with examples shown below

JUVENILE HORMONE REGULATION OF HMG-CoA REDUCTASE ACTIVITY AND TRANSCRIPT LEVEL IN ISOPRENOID AGGREGATION PHEROMONE PRODUCTION IN *IPS* SPP. BARK BEETLES

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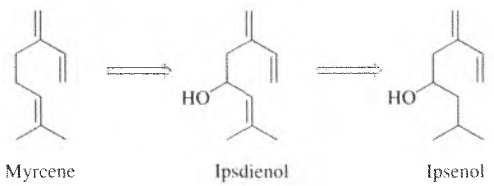
Origin of Isoprenoid Pheromone Components in Ips spp. The aggregation pheromone of the pine engraver beetle, *Ips pini*, consists primarily of enantiomeric blends of ipsdienol[1,2] and the achiral ketone lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one)[3]. Males of the California five-spined ips, *Ips paraconfusus*, produce both ipsdienol and ipsenol upon exposure to myrcene vapors[4]. The structural similarity between host-tree produced myrcene and ipsenol and ipsdienol and studies demonstrating that myrcene was converted to both ipsenol and ipsdienol[5] led to the conclusion that bark beetles obtained their pheromones only by selective hydroxylation of host monoterpenes[6].



However, alternative routes for *Ips* spp. pheromone biosynthesis exist. Studies with *I. paraconfusus*[7,8] questioned whether the volatile myrcene titer in its hosts could account for all of the ipsdienol and ipsenol produced by males. Subsequent biochemical studies revealed the occurrence of *de novo* production of monoterpene alcohol pheromones in some *Ips* spp. One study utilized the HMG-CoA reductase inhibitor compactin to offer strong indirect evidence that the production of the pheromone components ipsdienol and *E*-myrcenol in male *I. duplicatus* occurs *de novo via* the isoprenoid biosynthetic pathway[9]. Subsequent studies used radiotracer techniques to directly demonstrate *de novo* aggregation pheromone production in *I. pini* and *I. paraconfusus* males from [1-^{14}C]acetate and (*RS*)-[5-^{3}H]mevalonolactone (Fig. 1)[10,11].

To evaluate the relative contribution from *de novo* and host precursor-related pheromone biosynthesis, the masses and enantiomeric compositions of ipsdienol in California

populations of *I. pini* were examined from fed and unfed insects exposed to volatile myrcene. Unfed male *I. pini* produced only 8 ng/beetle of ipsdienol; fed males produced up to 5.426 ng/beetle [98.6%-(–)]; unfed-JH treated males produced up to 5.769 ng/beetle [89.4%-(–)]; whereas myrcene treated males produced up to 2.195 ng/beetle [44.9 to 61.3%-(–)][12]. Since California populations of *I. pini* produce and respond optimally to >95%-(–)-ipsdienol, the observation that myrcene-treated beetles produce nearly racemic ipsdienol suggests that although myrcene is readily hydroxylated by male *I. pini*, it is not metabolized to the behaviorly active pheromone blend. Unfed, JH-treated males produce enantiomeric blends resembling the naturally occurring composition, suggesting that the majority of pheromone produced arises from *de novo* synthesis and not from exposure to host myrcene[12].

Endocrine Regulation of Pheromone Production. Ivarsson and Birgersson[13] showed that JH analogs increased isoprenoid pheromone production in *I. duplicatus*. Likewise, topical application of JH III to unfed male *I. pini* resulted in a dose dependent increase in ipsdienol production[12].

Radiotracer studies were conducted with male *I. pini* using [1-^{14}C]acetate and (*RS*)-[2-^{14}C]mevalonolactone *in vivo* and (L)-[methyl-^{3}H]methionine *in vitro* to evaluate the relationship between feeding on host (*Pinus jeffreyi*) phloem, JH III biosynthesis, and *de novo* ipsdienol biosynthesis. The *in vivo* incorporation of radiolabeled acetate into ipsdienol by male *I. pini* increased with increasing topical JH III dose. The *in vivo* incorporation of radiolabeled mevalonolactone into ipsdienol by male *I. pini* was relatively high at all JH III doses, and did not significantly increase with increasing dose of JH III. Males fed on host phloem also significantly increased the in-

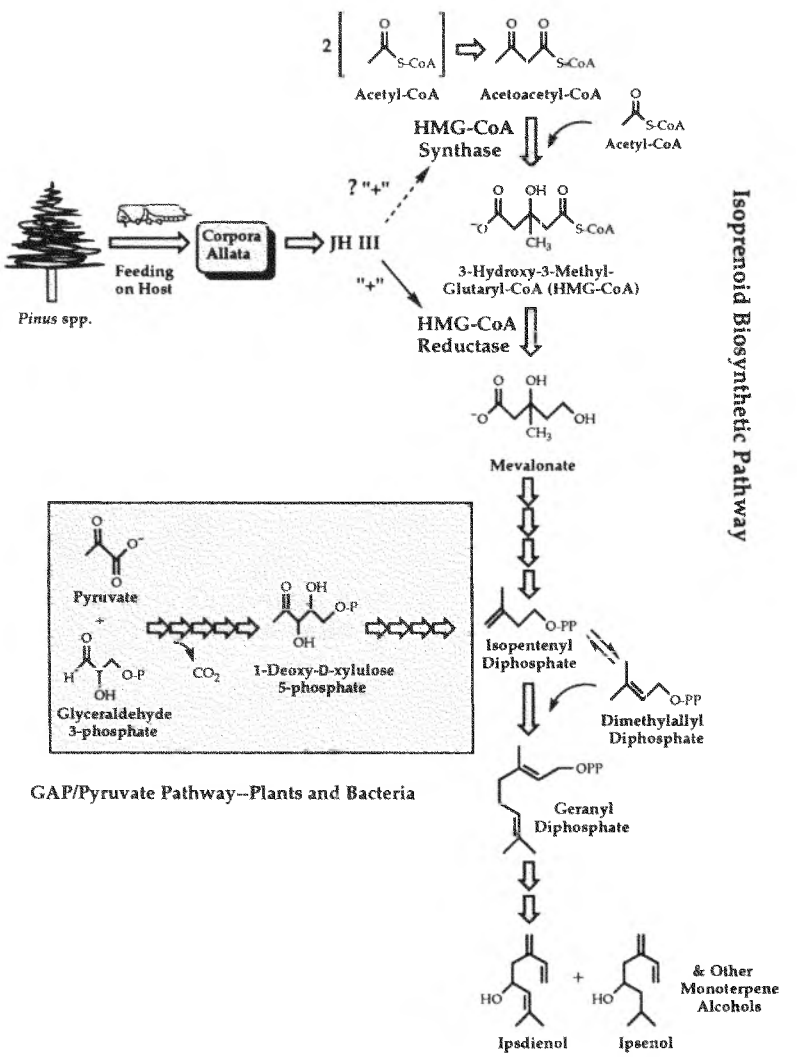


Fig. 1. Biosynthesis and endocrine regulation of pheromone production in *Ips pini*

corporation of ^{14}C-acetate into ipsdienol[11]. These data constitute direct evidence for the isoprenoid pathway in *de novo* ipsdienol biosynthesis, and indicate that JH III influences steps prior to mevalonate formation in this pathway.

HMG-CoA Reductase Activity in I. pini. HMG-CoA reductase (HMG-R) is a key regulatory enzyme in mammalian isoprenoid (cholesterol) biosynthesis, suggesting that HMG-R might also function as a key regulated enzyme in *de novo* monoterpenoid pheromone biosynthesis in *Ips* spp. Studies comparing HMG-R activity in fed, JH III-treated, and control male and female *I. pini* indicate that HMG-R activity is stimulated by feeding in females and by both feeding and JH III treatment in males[14]. This indicates that in the natural setting, feeding on host phloem stimulates JH III biosynthesis by the CA of male *I. pini*, with JH III specifically increasing the activity of HMG-R in the biosynthetic pathway (Figure 1). The following preliminary results suggest that JH III acts by inducing transcription of the *HMG-R* gene.

Induction of HMG-CoA Reductase Transcription in I. pini and I. paraconfusus. Studies using polymerase chain reaction (PCR) and northern blot analyses showed that topical application of JH III to male *I. paraconfusus*[15] and *I. pini*[14] increased the amount of HMG-R mRNA in a dose and time dependent manner.

Furthermore, this induction occurs in thoracic tissue of *I. paraconfusus*[16,17], and presumably *I. pini*. This finding is consistent with *in vitro* radiochemical data which shows that ipsdienone (a pheromone precursor) is produced in thoracic issue of male *I. paraconfusus*[16,17]. Work is currently underway using *in situ* techniques to pinpoint the exact location of pheromone synthesis. The current picture of pheromone regulation in *I. pini* involves the feeding-induced production of JH III, with JH III then inducing HMG-R transcription or increasing HMG-R mRNA stability in male thoracic tissue (Fig. 1).

An interesting and as yet unexplained difference exists between *I. pini* and *I. paraconfusus*. Feeding induces pheromone production in both species. However, in *I. pini*, JH induces HMG-R transcript levels, enzyme activity, pheromone production and the incorporation of ^{14}C-acetate into pheromone. In *I. paraconfusus*, JH induces higher transcript levels of HMG-R, but not increased enzyme activity nor does it induce high levels of pheromone production[14]. Thus, in *I. paraconfusus*, there may be an additional regulatory factor.

HMG-R and HMG-S in Dendroctonus jeffreyi. Recent work has resulted in the cloning and sequencing of both HMG-R and HMG-CoA synthase (HMG-S) in another bark beetle, *Dendroctonus jeffreyi*. In male *D. jeffreyi*, topical treatment with JH III results in labeling of frontalin by ^{14}C-mevalonate, the induction of frontalin production, and increased transcript levels of both HMG-R and HMG-S, suggesting that both of these enzymes are regulated at the transcriptional level by JH (ref.[18]).

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AN EFFICIENT APPROACH TO LOOK FOR THE MOST IMPORTANT FUNCTIONAL GROUPS IN BRASSINOSTEROIDS TO ELICIT ACTIVITY

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Brassinosteroids represent a class of endogenous plant growth regulators widely distributed in the plant kingdom. They possess high growth-promoting activity, and have been evaluated for use in improving crop yield, quality, stress tolerance such as chilling, salinity and drought, improving resistance to herbicidal injury and preventing pathogenic diseases[1].

The results reported by applying brassinosteroids over several plants have successfully demonstrated the suitability of such compounds for the enhanced production of field crops and vegetables. Although these findings are encouraging, fur-

ther detailed studies are required before the full potential use of brassinosterois can be realized.

Much effort is being done in the field of brassinosteroids from the physiological, biochemistry and molecular points of view as well as in the synthesis of new brassinosteroid analogs. But, the clarification of the structure-activity relationship, on which we are working intensively, is an important problem that remains to be solved and will help give a better understanding of the bioactivity and mode of action of such interesting compounds. Moreover, the search for new brassinosteroids with a good bioactivity-cost relationship is an active area for improving the benefits from these potent plant growth regulators in agriculture.

To achieve these goals we have developed a methodology based on the assumption that brassinosteroids act at molecular level through a receptor-ligand complex to regulate the expression of specific genes. Therefore, the active brassinosteroids should have a single defined three-dimensional "active conformation" able to bind to the receptor. On this active conformation, the atoms directly involved in binding with the brassinosteroid receptor ought to have the same spatial situation in all active molecules. Thus, the more complementary is the active conformation of a defined brassinosteroid to the three-dimensional structure of the receptor, the more active it should be.

Almost nothing is known about the receptor. Therefore, the establishment of a quantitative structure-activity relationship (QSAR) by considering the active conformation and the knowledge of which part of the brassinosteroid molecule are the most important ones in expressing activity would be useful for providing more information about the brassinosteroid-receptor binding at the structural level. This also should lead to a major advance in the understanding of brassinosteroid action. Moreover, this will enable us to predict the activity of new analogs and will eventually be of help in the design of the most suitable brassinosteroids for agriculture application with the best synthetic cost- activity ratio.

We have developed a methodology which allow us to define the active conformation of each active brassinosteroid[2]. Now, another interesting point to be determined concerns the type of interactions that can take place on binding and which of them contribute in a major way to the activity. In this sense, hydrogen-bonding interactions are being specifically analyzed. Therefore, if the region where the probability to form an H-bond is found, the interaction energy for these compounds is calculated, and some relationship is observed with the activity, it would indicate that this type of interaction may take place in the brassinosteroid-receptor complex.

Therefore, what we need to get this information is a broad set of brassinosteroids having sufficient structural modifications, their corresponding strictly homogeneous activity data with statistical parameters, the active conformation of each brassinosteroid and the so called GRID maps of each one of them, which simulate the H-bonding brassinosteroid-receptor interactions. The correlation between the activity and the GRID maps should allow us to get information about what are the essential groups on a brassinosteroid molecule to elicit activity.

The set of brassinosteroids of this lecture result from the combination of all of these kind of functionalities: As for the A ring, a diol α,α or β,β at C-2, C-3, and β-hydroxyl group at C-3 or no functionality at this ring. An A/B *cis* or *trans* ring junction. A lactone, isolactone, ketone, hydroxyl or ether functionalities at the B ring. A diol both *RR* and *SS* and different alkyl substituent at C-24 in the side chain.

Except brassinolide, all the set of brassinosteroids have been synthesized in our group[3-6].

The homogeneous activity data of the set of brassinosteroids were obtained using our modified rice lamina inclination test.

The active conformation of each brassinosteroid were obtained using molecular modeling techniques following a methodology developed by us[2].

Finally, the GRID maps were obtained following a procedure developed by Goodford[7] which can be used to simulate the different interaction between an active molecule and its receptor. Different probes has been used that simulate different type of interactions (H-bonding, steric, hydrophobic *etc.*). Among the probes tested, water has been chosen to calculate the interaction energy of H-bonding owing to its capability to act as both acceptor and donor of H-bonds.

The results currently obtained using water as probe will be analyzed by comparing different brassinosteroids containing only structural modifications in each one of the region studied: side chain, A-ring and B-ring. Moreover, how these results are in accordance with the ones obtained by comparing structurally brassinolide with the first brassinolide-inhibitor known at the moment called KM-01 will be discussed.

Based on the GRID methodology over the set of brassinosteroids studied the good correlation observed between the feasibility of H-bonding and the activity will be presented, suggesting that this type of interaction may take place in the brassinosteroid-receptor complex. Moreover, considering our findings when the KM-01 is involved in the study, the results obtained at present have allowed us to provide defined information about the areas of the molecule responsible for eliciting activity and those only responsible for binding with the receptor.

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STEREOCHEMISTRY AND MECHANISM OF THE CYCLIZATION/REARRANGEMENT CATALYZED BY ABIETADIENE SYNTHASE

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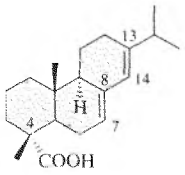
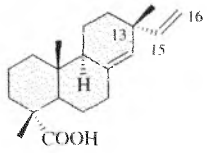
The resin acids comprise a family of perhydrophenanthrene-type diterpene carboxylic acids that occur widely in conifer oleoresin[1]. This viscous, sticky fluid, a mixture primarily of monoterpene hydrocarbons and diterpene resin acids, is a defensive secretion produced by pine, fir, spruce, and cedar trees in response to wounds caused by physical injuries, insects, birds, and diseases[2]. The resin acid constituents belong to either the abietane family of tricyclic diterpenes bearing an isopropyl substituent at C_{13} or to the pimarane family characterized by methyl and vinyl groups at C_{13} in both stereo-chemical variations. Relevant examples are abietic acid (*I*) and sandaracopimaric acid (*II*), both having the C_4 equatorial carboxyl group which typifies these natural products. The conjugated diene of abietic acid and the closely related congeners - levopimaric($\Delta^{8(14),12(13)}$ diene), palustric ($\Delta^{8,13}$ diene), and neoabietic($\Delta^{8(14),\ 13(15)}$ diene) acids - presumably participate in oxidative oligomerization and cross-linking reactions that occur as the volatile monoterpenes evaporate forming a hardened seal over the wound site. Other common pimarane constituents of conifer oleoresin are pimaric acid (13α methyl and 8(14) double bond) and isopimaric acid (13β methyl and 7(8) double bond).



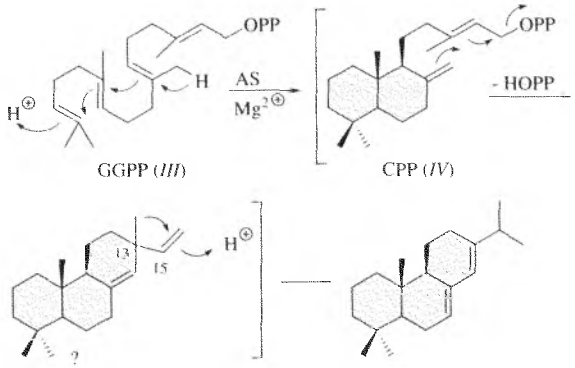
Abietic acid (*I*)



Sandaracopimaric acid (*II*)

In 1953 L. Ruzicka proposed that the pimaric and abietic acids are biogenetically related by a proton-induced rearrangement of the C_{13} methyl group of the former to C_{15} to form the isopropyl substituent and conjugated diene of the latter[3]. Wenkert and Chamberlin provided chemical precedent for this prescient proposal by acid-catalyzed rearrangements of both pimaric and isopimaric acids to abietic acid[4]. This lecture will present the results of a collaborative investigation on the stereochemistry and mechanism of the remarkable enzymatic cyclization/rearrangement(*III* → *VI*) catalyzed by recombinant abietadiene synthase (rAS) from grand fir (*Abies grandis*).

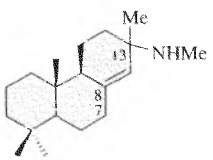
The native cyclase initially isolated from both wound-induced grand fir seedlings and lodgepole pine (*Pinus contorta*) stems catalyzes the conversion of the universal diterpene precursor (*E,E,E*)-geranylgeranyl diphosphate (*III*), to (–)-abietadiene (*VI*)[5]. The gene from induced grand fir encoding AS was subsequently isolated and the diterpene cyclase was expressed as a single 868-residue polypeptide including a putative *N*-terminal plastidial transit segment[6]. The recombinant enzyme *in toto* or in truncated form catalyzes the divalent metal ion-dependent cyclization of GGPP or (+)-copalyl diphosphate (*IV*) to abietadiene. Although none of the four plausible pimaradiene isomers was rearranged to abietadiene by rAS[7], the consistent appearance of 1–2% of sandaracopimaradiene(*Vb*) in the cyclization product may indicate an intermediate pimaradiene or pimarenyl carbocation



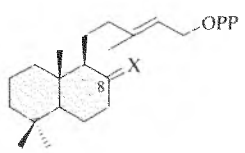
Pimaradiene (13α Me, *Va*)
Sandaracopimaradiene (13β, *Vb*)

Abietadiene (*VI*)

having the 13β methyl group and 8(14) double bond. The objectives of this collaboration were to obtain more definitive evidence about the nature and stereochemistry of the presumed pimaradiene or pimarenyl intermediate, to elucidate the stereochemistry of the rearrangement forming the isopropyl group by means of hydrogen isotope labelling, and to formulate a plausible mechanism for the coupled cyclization/rearrangement that gives rise to abietadiene.



13α Me (*VIIa*)
13β Me (*VIIb*)
13β Me (7,8-ene, *VIIc*)

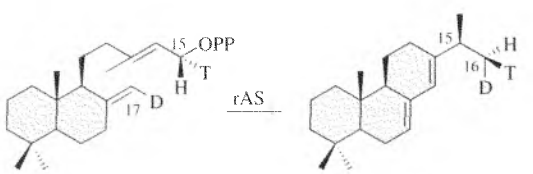


X = O (*VIII*)
X = α-OH, β-H (*IXa*)
X = β-OH, α-H (*IXb*)

Syntheses and enzymatic evaluations of three isomeric 15-aza-pimarenyl inhibitors (*VIIa,VIIb,VIIc*) and three 8-oxy-17-nor CPP analogs (*VIII* and *IXa*, *IXb*) were carried out at the University of Illinois. The higher potency of inhibitor *VIIb* in the presence of inorganic diphosphate anion indicates an intermediate or transition state resembling a sandaracopimara-8(14)-en-15-yl carbocation/PPi ion pair. This conclusion was reinforced by uncoupling the final cyclization and rearrangement by means of the 8α-hydroxy-17-nor CPP analog (*IXa*). The rAS-catalyzed cyclization of this intermediate analog afforded 17-nor manool with 13β methyl and

13α vinyl groups as the exclusive product. Similar rAS-catalyzed conversion of (15*R*)-[15-$^2H_1$] 8α-hydroxy-17-nor CPP to 17-nor manoyl oxide bearing deuterium in the 15*E* position revealed an *anti* SN′ ring closure for the oxy analog, and by inference for the CPP-sandaracopimarenyl cyclization.

We have shown previously by means of deuterium-labelling that the cyclization-rearrangement of CPP to abietadiene occurs with stereospecific "intramolecular" proton (or deuterium) transfer from the 17-*pro-E* position of the bicyclic intermediate to form the C_{16} *pro-S* methyl of the isopropyl group in the final product[7]. The stereochemistry of the proton incorporation at C_{16} has recently been elucidated by the double labelling experiment illustrated below. Enzymatic cyclization of (15*R*, 17*E*)-[15-$^3H_1$, 17-$^2H_1$]-CPP afforded [16-$^2H_1$, 16-$^3H_1$]abietadiene bearing a chiral methyl group of *R* configuration. It follows that the proton transfer to C_{16} and the following methyl migration to C_{15} occurs on the same face of the vinyl group of the putative sandaracopimaradiene intermediate, *i.e.* with overall *syn* stereochemistry.



[^2H, ^3H] CPP [^2H, ^3H] Abietadiene

The synthetic and analytical methods as well as the significance of the preceding and other results with regard to the mechanism of the enzymatic biosynthesis of abietadiene will be discussed

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VOLATILE ISOPRENOIDS THAT CONTROL INSECT BEHAVIOUR

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The sandfly, *Lutzomyia longipalpis* (Lutz and Neiva) (Diptera: Psychodidae) is the vector of the protozoan parasite *Leishmania chagasi* (Cunha and Chagas) (Kinetoplastida: Trypanosomatidae), the causative agent of visceral leishmaniasis in the New World. The male *L. longipalpis* congregate in leks where they release a sex pheromone from glands that can be seen as pale patches on the tergites of the abdomen[1]. Only the principal volatile component produced by the gland confers sex pheromone activity and is highly attractive to females[2]. Other minor components are not pheromonally active. This is confirmed by the presence of only one type of olfactory cell in the ascoid sensillum on the female antenna stimulated by the sex pheromone gland extract[3].

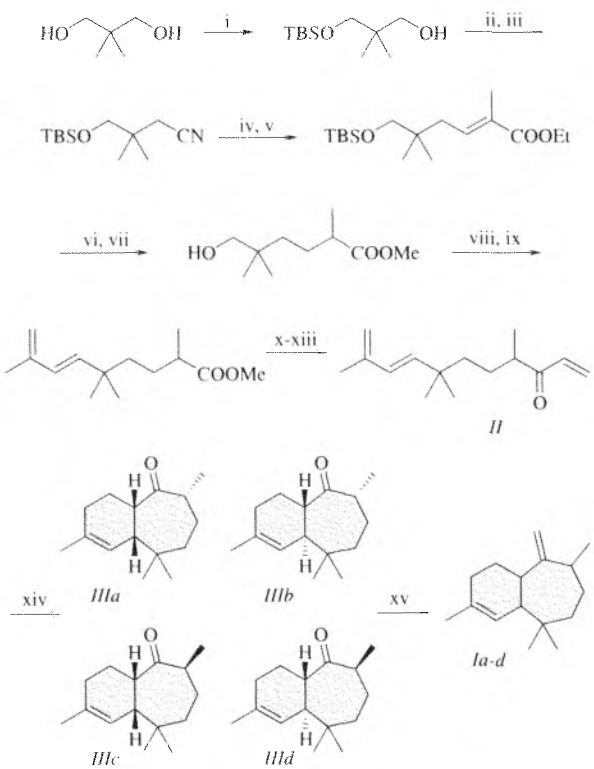
Lutzomyia longipalpis is a species complex and gas chromatography-mass spectrometry analysis[4] identified two populations based on differences in the unidentified components produced by the pheromone glands. One population produced a major component with a molecular weight of 218 and a fragmentation pattern consistent with a homosesquiterpene ($C_{16}H_{26}$) and the other produced a major component of molecular weight 272 as a diterpene ($C_{20}H_{32}$). It is now known that at least three chemically distinct members of the *L. longipalpis* complex exist[4] that are morphologically impossible to separate and which can only be identified by differences in the pheromone. Our work includes the identification, stereochemical characterisation and organic synthesis of two homosesquiterpenoid pheromones of two populations of *L. longipalpis*, one in Lapinha, southern Brazil[5a], and the other in Jacobina, northeastern Brazil[5b]. The work presented here describes that of the Jacobina population.

The major component from a crude extract from the pheromone glands *of L. longipalpis* from the Jacobina region was analysed by GC-MS of the natural and chemically modified pheromone and also NMR spectroscopy of the Florisil HPLC purified pheromone. Based on these results and the detection of α-himachalene as a minor component of the pheromone blend, the pheromone structure proposed was 2-methylene-3,6,6,9-tetramethyl-bicyclo[5,4,0]undec-8-ene *I* (3-methyl-α-himachalene)[6].

To verify the structure and determine which of the eight stereoisomers was the natural product, chemical synthesis was undertaken. The first aim was to generate all eight possible compounds for comparison to the natural pheromone and the second aim was to develop a synthetic strategy capable of generating enough material for field trials. The synthetic route employed was a result of an obvious Diels–Alder retrosynthesis[7] and construction of the linear substrate, which would hopefully cyclise to give all eight possible cyclisation products and thus all eight 3-methyl-α-himachalenes (Scheme 1). Cyclisation of the ketoene diene *II* in xylene under reflux gave a mixture of cyclic products that could be separated into enantiomeric pairs of diastereoisomers *IIIa–IIId*. Final conversion to the diastereomeric 3-methyl-α-himachalenes was

achieved using the Tebbe reagent. Assignment of the structures *IIIa–IIId* was by correlation and NOESY spectroscopy. Compounds *IIIa* and *IIIc* were designated as *cis*-fused by the ring junction protons showing a NOE to each other. The relative stereochemistry of the methyl group could be determined for *IIIc* by a NOE to the ring junction proton of C-1, a signal not seen for *IIIa*. The relative stereochemistries of the C-3 methyl group of *trans*-fused *IIIb* and *IIId* were also elucidated by NOE. A NOE was observed between the C-1 proton of *IIIb* and one of the C-6 methyl groups while the *other* C-6 methyl group showed a NOE to the C-3 methyl that therefore lies on the *opposite* face to the C-1 proton. The structures of *Ia–Id* were inferred from *IIIa–IIId* and in comparison with NMR data for natural α-himachalene[6].

Co-injection of natural product with the diastereoisomers, using high-resolution capillary GC on columns of different polarity gave peak enhancement only with diastereoisomer *Ic*. In the case of chiral GC, only one enantiomer of *Ic* enhanced the natural product peak. The ^{1}H NMR results reported[6], although incomplete, showed good agreement with only those



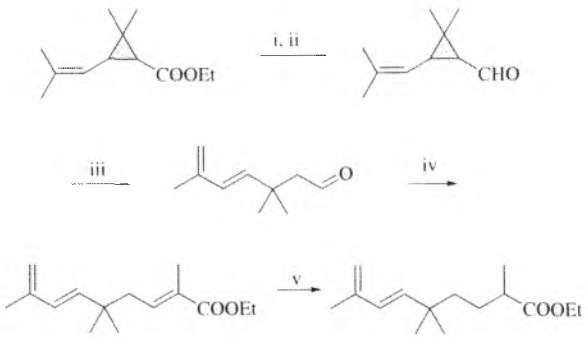
Scheme 1. Reagents and conditions: i, NaH (1.0 eq), TBSCl (1.1 eq), THF, 94%; ii, TsCl, C_5H_5N, $CHCl_3$; iii, NaCN, DMSO, Δ, 94% (2 steps); iv, DIBAL, CH_2Cl_2, H_3O^+, 67%; v, $CH_3C{=}(PPh_3)CO_2Et$, benzene, reflux, quant.; vi, Mg, MeOH, 88%; vii, HF_{aq}, CH_3CN, 97%; viii, Swern oxidation; ix, $Ph_3P{=}CHC(CH_3){=}CH_2$, THF, reflux, 56% (2 steps); x, $LiAlH_4$, Et_2O, 92%; xi, PCC, MS 4A, CH_2Cl_2; xii, $CH_2{=}CHMgBr$, THF, 70% (2 steps); xiii, Dess–Martin oxidation, 86%; xiv, xylene, reflux, 62%; xv, Tebbe reagent, quant.

for structure *Ic*. The MS spectrum for the natural product[6] was almost identical with that for *Ic*, with greater differences from those for *Ia*, *Ib* and *Id*.

Bioassays involving attraction of female *L. longipalpis* and conducted in a Y-tube olfactometer, showed that only diastereoisomer *Ic* and the natural pheromone extract were active and both showed high statistical significance. This was in agreement with the chromatographic and spectral data, giving the sex pheromone structure as (1*RS*,3*RS*,7*RS*)-2-methylene-3,6,6,9-tetramethyl-bicyclo[5,4,0]undec-8-ene (*Ic*). The *cis* stereochemistry of the natural product is analogous to that of natural α-himachalene[8]. The absolute stereochemistry of the pheromone is now under further investigation, although the bioassay results suggest no interference with biological activity by the sample containing both enantiomers.

The original synthesis of 15 steps requires the use of TBDMS protection. This has been reduced to 11 steps without protection and with subsequent cost reduction using the acid catalysed rearrangement of the aldehyde derived from ethyl chrysanthemumate (Scheme 2)[9]. This route implies possibilities of using a plant crop to generate the chemicals required for pheromone synthesis although the synthetic route must be further simplified. The Diels–Alder cyclisation step has also been modified to yield more of the desired diastereoisomer *IIIc*.

Sex pheromones are responsible for mate discrimination and thus can be part of the speciation process. Apart from their potential role in monitoring and controlling natural populations, sex pheromones can be a valuable aid to differentiation and classification of species where no morphological differences are discernible. Furthermore, they may be vital in work studying the spatial distribution of populations and their role as vectors of leishmaniasis.



Scheme 2. Reagents and conditions: i, $LiAlH_4$; ii, PCC; iii, TsOH, benzene, reflux, 39% (3 steps); iv, $CH_3C{=}(PPh_3)CO_2Et$; v, Mg, MeOH, 90% (2 steps)

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CHEMICAL DIVERSITY IN NATURE – ATTRACTANTS AND DETERRENTS

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The chemical diversity in Nature is almost endless. More than 100 000 low molecular weight natural products have been described and many more are to come[1]. The compounds are most probably essential in the life processes of the producing organism and specificity has been the driving force in evolution. I will by a few examples mirror the complexity as well as diversity of chemical compounds playing key roles in the plant and insect kingdom. The examples are mainly related to structural elucidations using NMR.

The problems with poisonous jellyfishes in tropical waters are severe and the plant *Ipomoea pes-caprae*, growing along the seaside, has been used in traditional medicine in Thailand for treatment of inflammations as well as dermatitis caused by the tentacles of jellyfish. The plant extract contained several active compounds interfering with the process of inflammation in different ways[2–4].

The antifeedant property of many plants is of great interest in order to substitute synthetic pesticides or herbicides with natural compounds. Four different isodrimeninols were isolated from *Polygonum glabrum* growing along the Nile in Sudan[5]. Several mushrooms are not attacked by insects or worms and have been the source for finding compounds with antifeedant activity. The *Lactarius* spp. show this property and have been a source for several interesting compounds mainly tested against storage pests[6]. Tropical plants from the Meliaceae family show resistance against insect attacks. A well-known example is azadirachtin from *Azadirachta indica*. The *Entandrophragma* spp. also contain rather complex substances with deterrent properties[7].

The fruit pulp of the Sri Lankan tree palmyrah, *Borassus flabellifer*, is part of the traditional diet. However, the bitter principle prohibits more extensive use of this very common native plant. The northern clones have been investigated[8], and, presently a new investigation of the saponins responsible for the bitter taste is under way for the southern clone[9].

The pine weevil, *Hylobius abietis*, is a pest in northern Europe of great economical importance. It mainly attacks young seedlings of spruce and pine. Under the threat of banning insecticides there has been a high research activity in this area. To understand the behaviour of the weevil as well as finding efficient antifeedants, studies have involved both attractants[10] and deterrents[11] of natural origin.

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STEROIDAL PLANT HORMONES: NEW SYNTHETIC APPROACHES AND BIOACTIVITY OUTSIDE PLANT KINGDOM

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The discovery of the hormonal functions of steroids in plants in addition to earlier known properties as the hormones of mammals, insects, and fungi shows their possible role as universal hormonal regulators, typical for most organisms inhabiting the Earth. Brassinosteroids (BS), which are steroidal plant hormones, demonstrate various kinds of regulatory activities in the growth and development of plants. Specific feature of BS is their ability to enhance plant resistance to unfavorable factors (stress, diseases, pollutants, *etc.*) and to improve the quality of crops together with crop yield increase. BS protective action is a result of stimulation of the natural protective forces of the plant itself. This can be reached by ap-

plication of very low doses, which are comparable with natural BS content in plants. All these are the reasons why BS are promising compounds for environmentally friendly approaches to plant protection and increasing crop yields. A new chemical for agriculture Epin, which includes one of the most active natural BS 24-epibrassinolide as an active ingredient, has been elaborated in our laboratory and officially registered in Russia, in Belarus, and in the Ukraine for application to cereals, legumes, potato, vegetables, fruits, ornamentals, and other plants.

Although BS are wide-spread in nature and are present in nearly every part of the plant, their content is extremely low. That is why efficient approaches to their large-scale preparation via chemical synthesis for the physiological studies and application in agriculture are under the elaboration in our laboratory for a number of years. Some new approaches to the synthesis of brassinolide and its analogs will be discussed.

Very interesting are the relations of steroidal hormones typical for certain organisms to the organisms that belong to other classes or kingdoms. Brassinosteroids from evolutionary and biochemical points of view seem to be promising in the search for regulatory functions in other types of organisms. The first data on the BS action in vertebrates and other animals will be presented.

STEROID BIOTRANSFORMATION

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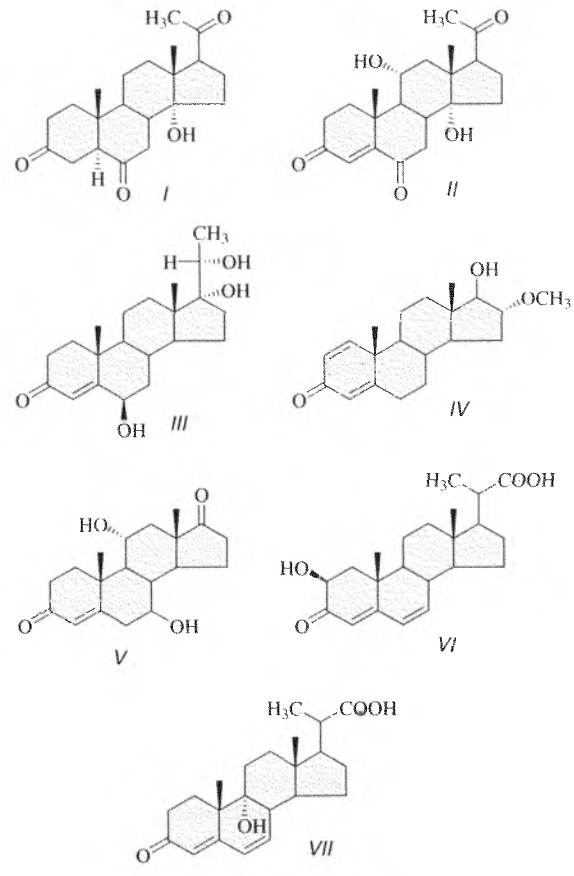
The importance of microbial biotransformation was realised for the first time in 1952 when Murray and Peterson of Upjohn Company patented the process of 11α-hydroxylation of progesterone by a *Rhizopus* species. Since then, microbial reactions for the transformation of steroids have proliferated, and specific microbial transformation steps have been incorporated into numerous partial syntheses of new steroids for evaluation as drugs and hormones. A variety of steroids are widely used as antiinflammatory, diuretic, anabolic, contraceptive, antiandrogenic, progestational, and anticancer agents as well as in other applications. Introduction of new steroids into commerce is now limited. However, our interest lies in improvement in the yields of desired metabolites as well as preparation of novel steroids which are difficult to synthesize by chemical means. The microbial strains used for our work were isolated from soil by enrichment culture technique using easily available steroids as the sole sources of carbon.

14α-Hydroxy pregnane and androstane derivatives are receiving attention for their useful biological activities. Fermentation of progesterone with a strain of *Bacillus* sp. yielded two novel 14α-hydroxy pregnanes *viz.* 14α-hydroxy-5α-pregnane-3,6,20-trione (*I*) and 11α,14α-dihydroxy-4-pregnene-3,20-dione (*II*) along with 14α-hydroxy-4-pregnene-3,20-dione and 11α-hydroxy-5α-pregnane-3,6,20-trione. The testosterone metabolites produced by the strain were identified as 4-androstene-3,17-dione, 17β-hydroxy-5α-androstane-3,6-dione, 14α-hydroxy-4-androstene-3,17-dione and 14α,17β-dihydroxy-4-androsten-3-one[1]. Fermentation of 17α-hydroxyprogesterone with the same organisms yielded a new pregnane analogue, 6β,17α,20α-trihydroxypregn-4-en-3-one (*III*) and two other metabolites[2].

A novel microbial transformation of 16-dehydropregnenolone by the strain of *Arthrobacter simplex* into a new androstane analogue, 17β-hydroxy-16α-methoxyandrosta-1,4-dien-3-one (*IV*) along with the isolation of an intermediate, pregna-1,4,16-triene-3,20-dione was achieved. The formation of this metabolite is a multistep process involving a microbial generation of a methoxy group from a double bond transformation[3,4].

Cells from microorganisms dried with acetone are useful for special preparations, although this process does not compete with vegetative cell fermentations for manufacture. Usual fermentation of androst-4-ene-3,17-dione (AD) with a fungal strain, *Aspergillus fumigatus* furnished a new dihydroxy, 7β,11α-dihydroxy AD (*V*) and another steroid 6β,11α-dihydroxy AD along with minor metabolites, androta-1, 4-diene-3,17-dione (ADD), Δ^1-testosterone and testosterone. However, fermentation of AD with acetone-dried cells of the strain gave ADD as the major product and testosterone and its Δ^1 analogue as minor ones[5]. Apparently in acetone - dried cells, the hydroxylase enzymes are in-



activated, but the dehydrogenase and reductase enzymes remain active.

Selective 1-dehydrogenation of progesterone by the strain of *A. fumigatus* was achieved by fermentation of progesterone with acetone-dried cells of the fungus in the presence of β-cyclodextrin[6]. The results demonstrated that in acetone-dried cells, the hydroxylase and Δ^4-reductase enzymes are inactivated. The activity of the enzyme system responsible for the degradation of the side chain of progesterone is inhibited in the presence of β-cyclodextrin. Inhibition of certain enzymes in presence of cyclodextrin is a phenomenon of considerable interest.

Microbial productions of biologically important 15β-hydroxytestosterone[7] from testosterone and 7β,15β,17α-trihydroxyprogesterone[8] from 17α-hydroxyprogesterone as single isolable products in appreciable yields by fermentation with the strain, *A. fumigatus* were achieved.

A novel metabolite, 2β-hydroxy-3,12-dioxo-23,24-dinorchola-4,6-dienoic acid (*VI*) was produced along with a few other metabolites by transformation of cholic acid by *Arthrobacter simplex*. The pathway of formation of these metabolites has also been rationalised[9,10].

A gram positive bacteria was isolated using cholic acid as the sole source of carbon. It does not consume sugars but utilizes fatty acids. An interesting new metabolite, 9β-hydroxy-3,12-dioxo-23,24-dinorchola-4,6-dienoic acid (*VII*) was produced as a single isolable product by fermentation of cholic acid with this strain[11].

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THOUGHTS ON THE BIOSYNTHESIS OF THE TRITERPENOID AZADIRACHTIN

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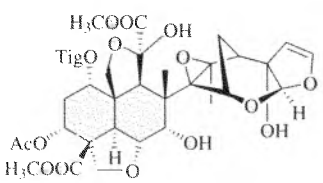
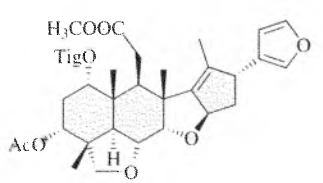
A few species of the Meliaceae produce a group of highly transformed and oxygenated triterpenoids, the meliacins, of which the most interesting is azadirachtin (*I*), because of its potent antifeedant and insecticidal effects[1]. Azadirachtin, and a few of its close relatives, from *Azadirachta indica* A. JUSS. (the neem tree) and *A. excelsa* (JACK.) (the sentang or marrango tree), are among the most highly oxygenated triterpenoids yet isolated. The biosynthetic pathway by which it and its relatives are formed is therefore of special interest. Analytical methods for the separation of many of the important products are now available, and the conditions for culturing neem cells in disperse medium to produce azadirachtin are now known. A consideration of the biosynthetic pathways is a valuable preliminary to planning biosynthetic experiments.

At least 128 triterpenoids have been isolated from various parts of *A. indica* and their structures permit an informed speculation about much of the biosynthetic route. Some of these proposals have already been made elsewhere[2-4]. Moreover the compounds produced in tissue cultured cells, their sequence of production, and attempts to convert salannin (*II*), a major product in neem seeds, into further products with a crude enzyme preparation give further clues to the biosynthetic pathway.



(*I*) Azadirachtin

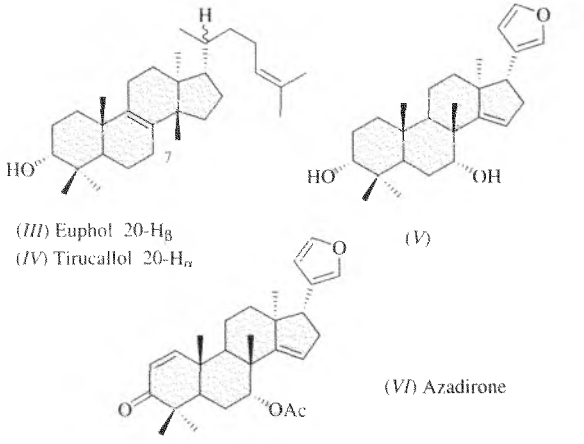


(*II*) salannin

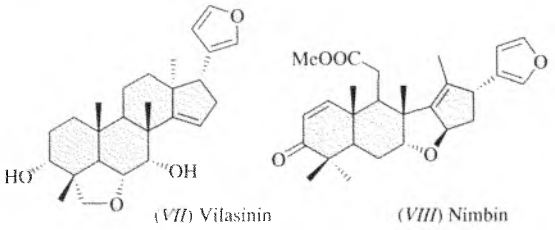
A survey of plants containing triterpenoids derived from euphol (*III*) and tirucallol (*IV*), and the isolation of one tirucallol derivative and five Δ^7-tirucallol derivatives from neem suggests the cyclized squalene is a tirucallol type. Rearrangement to an *apo*-tirucallol then follows for most of the molecules being processed. Loss of four carbon atoms from the side chain and formation of the furan ring, characteristic of the limonoids, is an early process. Compounds with the structure (*V*) are called meliacins. Some 126 such meliacins

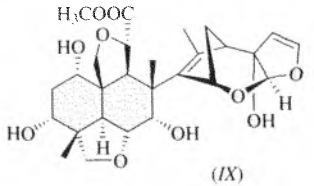
are known, of which azadirone (*VI*) and 20 related compounds are the closest examples.

Many of the compounds isolated appear to be dead ends of metabolism, including a few D-ring opened products and expanded A-ring substances. Most of the triterpenoid material is next converted to vilasinin-type structures with a C-28 to C-6 ether linkage (*VII*). The most abundant compound found among the neem triterpenoids, salannin (*II*) and over 60 others have this element of structure. The next step is the cleav-



age of ring C, either with formation of a C-7 to C-15 ether linkage as in nimbin (*VIII*), another major product, with 10 similar substances, or with formation of the C-15 to C-21 ring. The route proposed for the cleavage of the C-ring between C-12 and C-13, and formation of the C-7 to C-15 ether is supported by the existence of five substances oxygenated at C-12 and a further five compounds which can be regarded as half-way stages in this ring-opening. Breaking of the C ring is followed by oxidation at C-11, with involvement of C-19 to form ether, hemiacetal or lactone rings, but only in those compounds with the C-15 to C-21 ring.





A proposal for formation of the C-15 to C-20 ring of hypothetical structure (*IX*) (with C-28 un-oxidized it is called a meliacarpin) is more difficult because of the lack of helpful intermediates. Twenty three compounds of this type (*IX*), with C-19 and C-11 in various stages of oxidation have been isolated from neem, and are found only in the genera *Melia* and *Azadirachta*. Finally the oxidation of the C-28 methyl group is found only in the *Azadirachta*. It is interesting to note that in tissue cultures of neem cells that have been subcultured repeatedly, the ability to perform this last oxidation is lost first.

In spite of the large number of triterpenoids isolated, and at least 50 of these have been tested biologically against insects, with particular attention being given to those close to azadirachtin, little can yet be said about structure activity relationships in the group[3,4]. It would appear that as changes are made to the azadirachtin structure, either to add on groups or to reduce some of the extant functions, activity is slowly decreased as more changes are made.

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VERSATILE REACTIONS CATALYZED BY ADRENOCORTICAL CYTOCHROME P450 (11β) ISOFORMS AND THEIR IMPLICATION IN GLUCOCORTICOID AND MINERALOCORTICOID BIOSYNTHESIS

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The final step of biosynthesis of glucocorticoids such as corticosterone (B) and cortisol (F) is the 11β-hydroxylation of 11-deoxycorticosterone (DOC) and 11-deoxycortisol (DOF), respectively (Fig. 1). An enzyme that catalyzes the 11-hydroxylation of DOC was first purified to homogeneity from bovine adrenocortical mitochondria in 1978, found to belong to a family of cytochrome P450s, and hence named cytochrome P450(11β) (CYP11B)[1] (see refs[2,3] for review). It was soon established that the enzyme also catalyzes the 18-hydroxylation of steroids. The results of studies under-

taken to characterize the enzymatic activities of bovine CYP11B suggested two logically possible metabolic pathways starting from DOC in the adrenal cortex. The first is one in which DOC is hydroxylated at C-11 and B so generated is then hydroxylated at C-18 to produce 18(OH)B. The second possible pathway is one in which DOC is first hydroxylated at C-18 and the product, 18(OH)DOC, is then converted to 18(OH)B through the 11β -hydroxylation. All the four reactions have been shown to occur in the reconstituted system of bovine enzyme. Steroids other than the above-mentioned four were found in the incubation mixture of bovine CYP11B with either 18(OH)DOC or DOC. Further analyses of these steroids resulted in disclosing more versatile nature of the enzyme; its ability to catalyze the 19-hydroxylation of steroids[4]. It is now known that in the reconstituted system of bovine CYP11B DOC is sequentially converted to 19(OH)-, 19-oxo-,

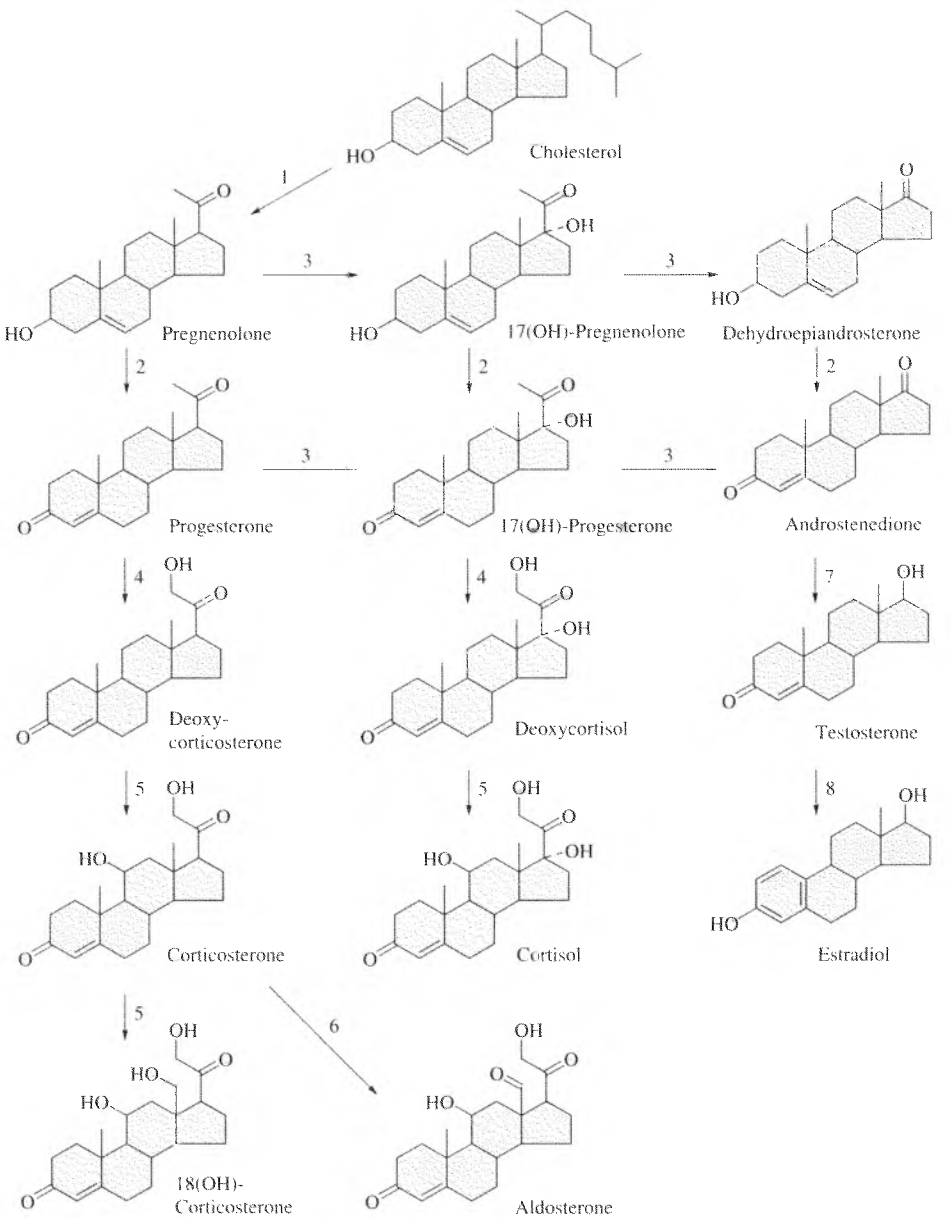

Fig. 1. Biosynthetic Pathways of Steroid Hormones. Numbers on the arrows denote the enzymes catalyzing the reactions. 1. Cytochrome P450(scc); 2. 3β-Hydroxysteroid dehydrogenase; 3. Cytochrome P450(17α, lyase); 4. Cytochrome P450 (c21); 5. Cytochrome P450(11β): CYP11B1; 6. Cytochrome P450(aldo): CYP11B2; 7. 17β-Hydroxysteroid dehydrogenase; 8. Cytochrome P450(arom)

and finally 19-oic-DOC. The latter, stored at -20 °C, was nonenzymatically decarboxylated to produce 19-nor-DOC, a potent mineralocorticoid[5,6].

Enzymatic nature of biosynthesis of aldosterone (ALDO), the most potent mineralocorticoid, had long been elusive until Wada *et al.*[7,8] demonstrated that the purified bovine CYP11B produced ALDO from B. At first this finding was received by skepticism, because ALDO is known to be exclusively produced in the zona glomerulosa of adrenal cortex whereas CYP11B is present in the zonae fasciculata and reticularis as well as the zona glomerulosa of bovine adrenal cortex. In the meantime rat was shown to have two CYP11B-related proteins, both immuno-crossreacted with a monoclonal antibody raised against bovine CYP11B, but different from each other in their apparent molecular weights; one present in the zona glomerulosa of K^+-treated rat adrenal, a possible candidate for ALDO synthase, and the other, in the zonae fasciculata and reticularis of normal rat, possibly 11β-hydroxylase[9].

Gene-cloning technology has rapidly advanced the knowledge concerning the molecular nature of these enzymes. Three CYP11B isoforms whose primary sequences were slightly different from each other were identified in bovine adrenal glands. All the three were shown to have not only 11β-hydroxylation and 18-hydroxylation activities for DOC, but also ALDO synthetic activity[10]. In contrast to cattle, three CYP11B-related enzymes possessing distinct activities have been isolated from rat. CYP11B1 catalyzes 11β-, 18- and 19-hydroxylation of DOC to produce B, 18(OH)DOC and 19(OH)DOC, respectively, but not ALDO production. CYP11B2 catalyzes both 11β- and 18-hydroxylations to produce B, 18(OH)B and 18(OH)DOC and also catalyzes ALDO

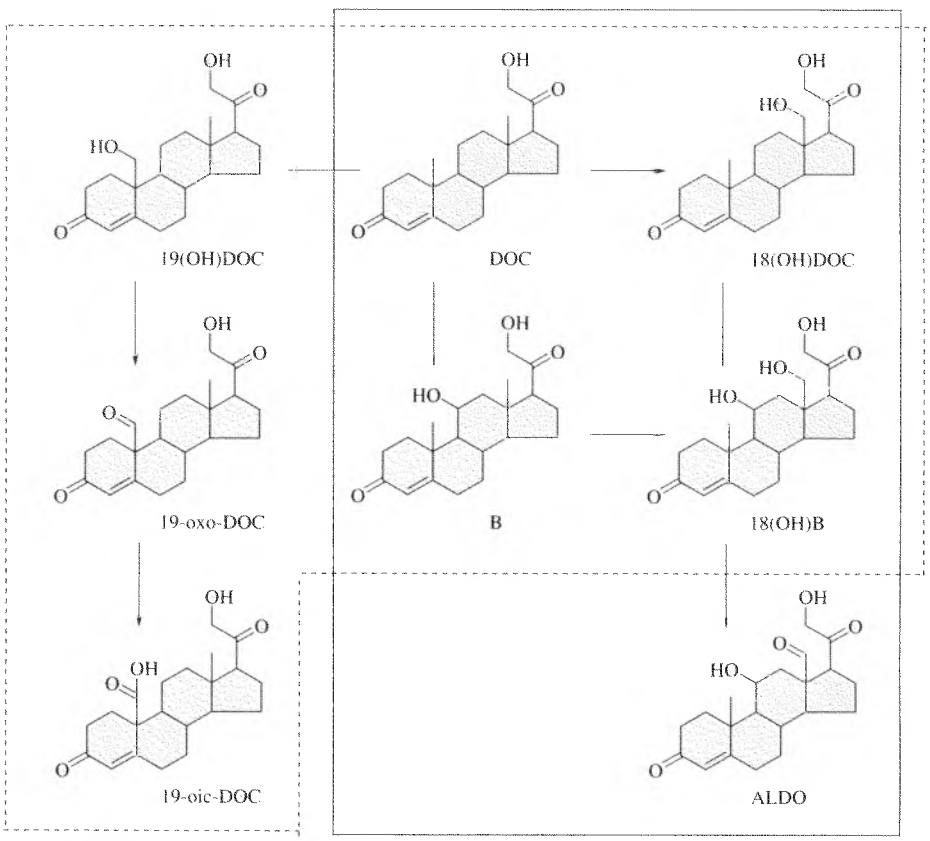

Fig. 2. Steroid Hormone Production from DOC mediated by CYP11B1 and CYP11B2

synthesis, but does not carry out 19-hydroxylation[11-13] (Fig. 2). CYP11B3, recently cloned from rat, is closely similar to CYP11B1, and has not been enzymatically characterized yet.

Summarizing these results and those reported on the other animal species, we can conclude that the adrenal glands of frog[14], cattle, pig[15] and sheep contain a single enzyme having activities to produce B, 18(OH)DOC, 19(OH)DOC and ALDO from DOC. We have proposed to call this enzyme CYP11B0. On the contrary, those of rat, mouse[16], hamster, guinea pig and human[17] contain at least two distinct CYP11Bs; one, CYP11B1, has the activities to produce B, 18(OH)DOC and 19(OH)DOC, but not ALDO, and the other, CYP11B2, has the activity to produce ALDO (Fig. 3).

Based on the accumulating knowledge of the primary sequences of CYP11Bs of various animal species, attempt has

been made next to investigate the structural basis of versatile activities catalyzed by these enzymes. To analyze the structure/function relationship of these enzymes, we have mainly focused on rat CYP11B1, because Dahl's salt-resistant (DR) rat expresses CYP11B1 containing five missense mutations that has the steroid hydroxylation activity distinct from the

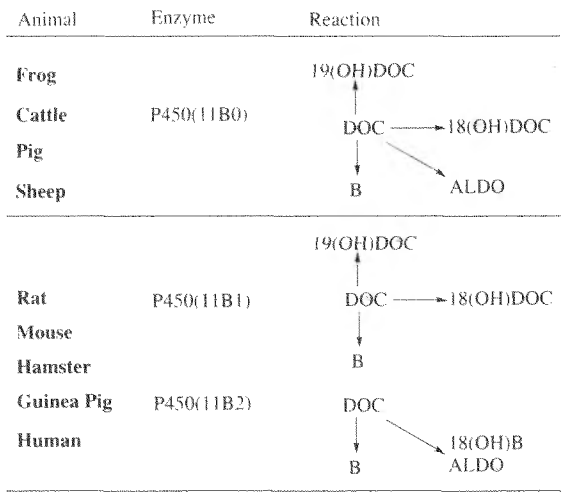

Fig. 3. CYP11Bs of Various Animal Species

wild-type enzyme[18]. Thus DR-CYP11B1 contains R127C, V351A, V381L, I384L and V443M alterations. Both the wild-type and DR-CYP11B1s were successfully expressed in *E. coli*. Steroid 11β-hydroxylase activity observed with DR-CYP11B1 was similar to that of wild-type CYP11B1, while 18-hydroxylase activity of DR-CYP11B1 was lower than that of wild-type CYP11B1. Mutant CYP11B1s containing a single or a double amino acid substitution associated with DR-CYP11B1 were tested for the enzymatic activity. The result was that a double mutant CYP11B1 with V381L and I384L showed 18-hydroxylase activity at a similar low level to that of DR-CYP11B1, suggesting that these two amino acid substitutions account for the decreased 18-hydroxylase activity[19]. Further study on the similar track may lead to clearer understanding of the mechanism of action of these interesting family enzymes.

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A NEW APPROACH TO THE INTERPRETATION OF ORGANIC MASS SPECTRA

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Almost any type of mass spectrometric measurement should be provided by skilful people but the interpretation of mass spectra is the real queen of mass spectrometry.

So called soft ionization techniques, *e.g.* fast atom or ion bombardment (FAB, FIB), electrospray (ESP), atmospheric pressure chemical ionization (APCI) and matrix-assisted laser desorption/ionization (MALDI), give mainly an information on molecular weight and on large parts of the molecule and there is rather difficult, with some exceptions *e.g.* peptides and carbohydrates, to define and apply general fragmentation rules.

A main effort was focused on finding fragmentation rules and interpretation procedure for electron ionization spectra because, in general, they show many peaks of structurally important ions, are very reproducible and the fragmentation is in an accordance with general accepted rules.

Several good books on mass spectra interpretation already exist but in the most cases are dedicated to an explanation of mass spectral fragmentation and an interpretation of the spectra of known compounds while the spectra interpretations of unknown compounds are simplified to a few basic steps. Unfortunately there is a great difference between following the formation of intense peaks from a known structure and identifying an unknown structure from a group of numbers on a spectrum. A spectrum is a complex of peaks of ions and only some of them are structurally important. During an interpretation procedure one must distinguish structurally important ions, determine their structure and correlate them each other to find a final structure of a molecul.

A new approach to the interpretation of organic mass spectra consists in using so many discrete data obtaining steps as was possible to put together in order to obtain so much information as possible. The all data obtained are correlated continuously and finely. A number of steps is a maximum and not all of them (depending on a type instrument used) is ever possible to use. A new approach was also used to calculate a plausible elemental composition of any ion without a high resolution measurement.

General procedure for the interpretation of electron ionization mass spectra is as follows:

1. Study of the sample history and information from other spectroscopic and chemical methods.
2. Make the decision if it is the spectrum of a pure compound or a mixture (use also re-constructed fragmentograms).
3. Use a mass spectra data base available and make notes on structures and important peaks of reference compounds.
4. Identify the peak of molecular ions and according to its intensity and intensities of other important peaks consider the stability of a molecule.
5. Use some type of a soft ionization technique (*e.g.* CI, FAB, ESP, APCI) when molecular ion was not identified.
6. Apply the Nitrogen rule.
7. Look for characteristic series of ions to determine a type of compound measured.
8. Select the structurally specific ions.
9. Use natural isotopic abundance to identify such elements like Cl, Br, S, B, Si.
10. Mark odd-electron ions.
11. Determine the elemental composition of both the molecular ion and structure important ions using one of the following possibilities:
a. High resolution measurement.
b. Natural isotopic abundances (see bellow)[a].
c. Calculation of plausible possibilities (see below)[b].
12. Calculate the Double bond equivalent (DBE) (number of rings and double bonds) for all structurally important ions and try to draw their structure. (DBE can also distinguish odd-electron ions from even-electron ions.)
13. Determine the number of active hydrogen atoms using C_2H_5OD or CH_3OD.
14. Find relationships between ions and draw a fragmentation map:
a. Refuse unlikely differences.
b. Look for differences which mean the loss of neutral fragments.
c. Use re-constructed fragmentograms.
d. Apply mass-analyzed ion kinetic energy spectroscopy (MIKES), collisionaly activated dissociation (CAD) with linked or combined scans when available.
15. Apply the Even-electron rule.
16. Use a derivatization if necessary (*e.g.* methylation, silylation, hydrogenation, hydrogenolysis, transesterification, specific reaction for the determination of double bond positions).
17. Study the fragmentation of reference compounds:
a. Study published fragmentation (remember for some compounds there are complete fragmentation pathways).
b. Measure reference compounds and study their fragmentation.
18. Take in to account that stereochemistry plays only a very limited role in basic fragmentation pathways.
19. Apply the Stevenson–Audier's rule when data available.
20. Apply the Field's rule when data available.
21. Use Self-training interpretative and retrieval system (STIRS) when on-line access available.
22. Use other spectroscopic methods - NMR, IR, UV - to complete structural information.

[a]Note to item 11b: Use of natural isotopic abundances:

Organic molecules consist of elements which, in the most cases, have isotopes whose abundance is in accordance with statistically random occurrence. In mass spectra, the detected ions correspond to a sum of mass of both the most abundant naturally occurring isotopes of various atoms that form their structures (*e.g.* molecular ion) and also one or more less abundant isotopes (isotopic ions).

Numerical values for a combination of isotopes are calculated using binominal expansion $(a + b)^n$, where a and b represent relative amount of isotopes (*e.g.* for chlorine it is 3 and 1) and n is a number of atoms in an ion.

In carbon, the ratio of the first two members of binominal expansion (I_A, $I_{(A+1)}$), for any number of carbon atoms, is always $89.9 : X = I_A : I_{(A+1)}$, where X is a number of carbon atoms in the ion. From this equation $X = (I_{(A+1)}/I_A) \times 89.9$, which means two things:

a. If the ratio of intensities of isotopic and monoisotopic peaks is multiplied by 89.9 the number of carbon atoms in the ion with an accuracy ± 1 is obtained. (Limitation: An ion may contain only atoms of C, H, O, Cl, Br, F, I, max. 2 atoms of N and no elimination or addition of H atom may occur.)
b. If the number of carbon atoms in an ion is 90 then the first isotopic peak has the same intensity as the monoisotopic peak. When the number of carbon atoms increases above this limit then the first isotopic peak becomes to be more intensive then the monoisotopic peak.

[b]Note to item 11c: Calculation of plausible elemental composition of ions based on *m/z* value and correlation of compositions only.

Even the accurate mass from a high resolution measurement is not available and an information from isotopic abundances is not useful there is still possibility to calculate a few plausible elemental compositions.

Calculation procedure is as follows:

It is always necessary to start with an elemental composition for hydrocarbon. To make this it should be due *m/z* value divided by 14 (CH_2). If the number obtained is not integer one adds or subtracts due number of hydrogen atoms to get right *m/z* value. It does not matter if the first composition calculated is not real (usually to many hydrogen atoms) because it is just a starting composition. Then when one atom of oxygen could be added it is necessary to subtract one atom of carbon and four atoms of hydrogen. The subtraction of one atom of carbon and two atoms of hydrogen is due to nitrogen. In this manner is it possible to continue in order to obtain more possibilities. For each elemental composition calculated DBE is also immediately calculated to get some idea how the ion looks like. The all elemental compositions which are calculated for several ions are correlated to choose the right compositions only. When the studied ion contains elements like Cl, Br, S, Si or B the weight of this element should be subtracted from an original *m/z* value and then calculation continues in the same manner as earlier.

The described procedure is demonstrated on *m/z* 157:

The described procedure is demonstrated on *m/z* 157:

157 : 14 = 11.2 → $C_{11}H_{25}$ starting composition

Addition of O	DBE	Addition of N	DBE	Addition of O+N	DBE
$C_{10}H_{21}O$	0.5	$C_{10}H_{23}N$	0	$C_9H_{19}NO$	1
$C_9H_{17}O_2$	1.5	$C_9H_{21}N_2$	0.5	$C_8H_{15}NO_2$	2
$C_8H_{13}O_3$	2.5	$C_8H_{19}N_3$	1	$C_7H_{13}N_2O_2$	2.5
etc.		*etc.*		*etc.*	

In highly unsaturated compounds:

Composition	DBE
$C_{12}H_{13}$	6.5
$C_{11}H_9O$	7.5
$C_{11}H_{11}N$	7
etc.	

In chlorine containing compounds:

157 – 35 = 122 : 14 = 8.7

Composition	DBE
$C_9H_{14}Cl$	2.5
$C_8H_{10}ClO$	3.5
$C_8H_{12}ClN$	3
etc.	

P1
13α-ESTRA-1,3,5(10)-TRIENES – SYNTHESES AND CONFORMATION

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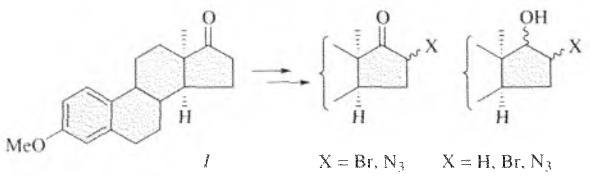
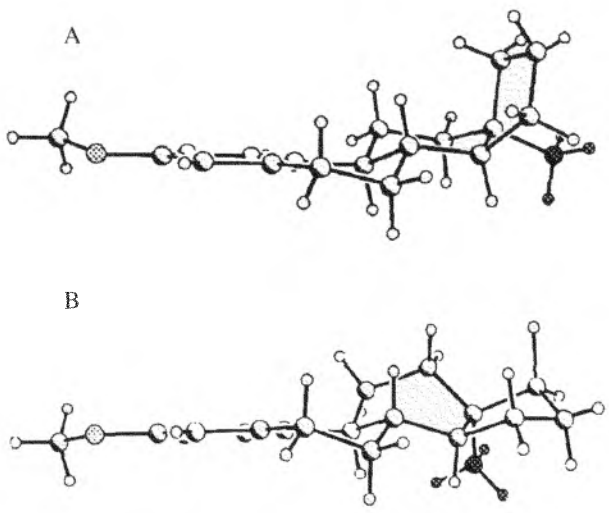
13α-Estra-1,3,5(10)-trienes, steroids with a *cis*-hydrindane system, are now available from the natural 13β-estra-1,3,5(10)-trienes using a convenient one-step isomerization procedure. Starting with 3-methoxy-13α-estra-1,3,5(10)-triene-17-one (*I*) syntheses of compounds with a 17-oxygen function and 16-bromo- or -azido substiuents are described.



I X = Br, N_3 X = H, Br, N_3

16α; 16β 16α, 17α; 16β, 17β
16α, 17β; 16β, 17α

Conformational investigations with x-ray analysis and ^{1}H-NMR spectroscopy show that aside from steroids with the typical rigid conformation (A) there are also steroids with an unusual flexible conformation (B) (twist-boat conformation of the C-ring and pseudorotation of the D-ring). The substituent patterns, leading either to rigid or flexible conformation, have been determined.



Compounds having a 17α-hydroxy or a 17-keto group possess the rigid conformation, while the 17β-hydroxy compounds as well as compounds with an epoxy group in the D-ring have the flexible conformation. On the basis of the substituent pattern compounds with either of these conformations can be intentionally synthesized. These compounds are then all potentially useful for comparison of receptor-mediated biological activities and as starting material for vicinal amino alcohols and derivates with metal ion binding activities.

P2
A NEW PINANE DERIVATIVE WITH C_2 SYMMETRY. PART I. 10,10′-BIISOPINOCAMPHEOL-SYNTHESIS, CRYSTAL AND MOLECULAR STRUCTURE

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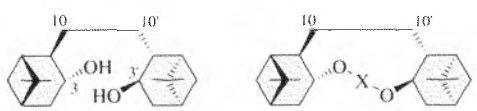
α-Pinene, available in both optical forms, is a starting material for the preparation of chiral auxiliaries, which can be successfully used in asymmetric synthesis. A new C_2-symmetric potential chiral ligand with pinane skeleton is presented.

From α-pinene with low optical purity (≈70%) we prepared mixture of 1,5-dienes *via* myrthenyl bromide. The major product (10,10′-bi-α-pinene) is a precursor of 10,10′-bi-isopinocampheol *I*. Compound *I* was isolated from the mixture by column chromatography. After recrystallisotion it twice from methyl–cyclohexane, optical pure crystals of *I* were obtained, m.p. 152 °C, $[\alpha]_D^{20} = -32.8°$ (c = 3.5, $CHCl_3$), (for details see[1]).



I *II*

The title compound *I* has two isopino-campheol fragments joined together by $C^{10}-C^{10}$ bond, therefore it is a 1,6-diol having a C_2-symmetry axis and is able to form nine-membered ring complex *II*.

X-ray structure analysis of *I* shown two independent molecules in unit cell (space group $P2_1$, for details see[2]. The two independent molecules of the same enantiomer of *I* are almost identical (Fig. 1) differing only in the position of two pair of H-atoms: O31-H1 vis O33-H3 and O32-H2 vis O34-H4.

However the molecule of *I* loses the C_2 symmetry in the crystal (Fig. 1) and each particular molecule is spiral down x-axis In whole crystal both molecules form double helix forward y-axis (Fig. 2). Inside that helix, the molecules are

joined by infinitive eight-membered chain of O34-H4.. O32-H2..O31-H1..O33-H3. As a certain circumstance for this chain, corresponding H-atoms from independent molecules should be unlike. Potential solvent volume inside the helix equals about 60 Å (ref.[3]).

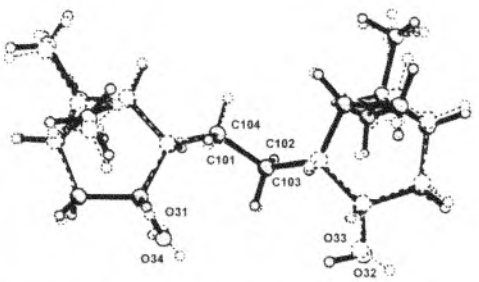


Fig. 1. Fitting of two independent molecules of *I*

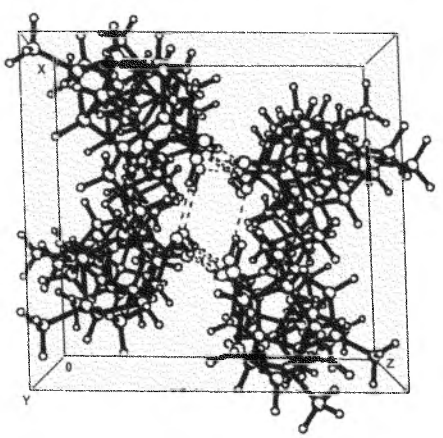


Fig. 2. Packing of the unit cell

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P3
A NEW PINANE DERIVATIVE WITH C_2-SYMMETRY. PART II. DI [3α -(2α-HYDROXY)PINANE] AMINE

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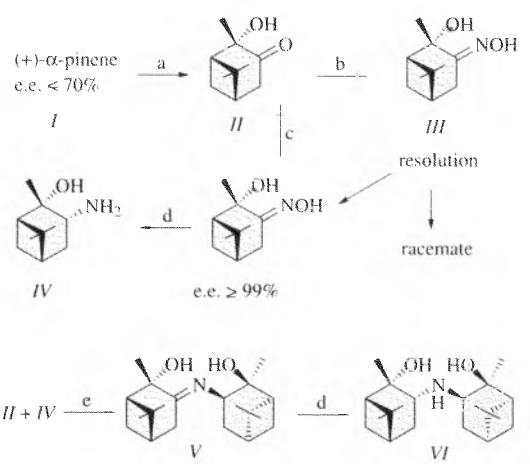
Whether the chiral auxiliary could be useful in asymmetric synthesis is often determined by the availability of optically pure synthetic material from raw substances with low enantiomeric excess. Expecting that Schiff bases of 2α-hydroxypinan-3-one would behave analogously to previously observed[1] (different crystals of optically pure form and racemate), we decided to work out a convenient method of obtaining an optically pure oxime from ketone *II* (2α-hydroxypinane-3-one) of e.e. ≤70%.

Hydroxyamine *IV*, obtained from oxime *III* (ref.[2]), turned out to be a good catalyst for reduction of prochiral ketones with hydroboron[3]. For us it (hydroxyamine *IV*) was a compound from which we could obtain a heavily sterically hindered amine *VI*. We came to a conclusion, that the racemic oxime, crystallizing from overcooled chloroform solution in the shape of prisms, has m.p. = 130–131 °C, which is significantly higher than this of optically pure oxime, crystallizing in the shape of needles (118–119 °C)[2]. Crystals of the racemate, practically insoluble in boiling hexane, can be easily separated and the oxime crystallizing from hexane has optical purity greater than 90%. The optical purity can be improved to e.e. >99% under conditions presented by Shioiri[2b] or by crystallization from chloroform and washing with hexane. The synthesis of *VI* is illustrated below.

The steric hindrance causes the reaction of forming Schiff base from hydroxypinanone *II* and hydroxyamine *IV* proceeds slowly and modification of catalyst (BF_3·Et_2O) by adding silicagel is required. The last step, the formation of *V* to *VI* with LAH proceeds easily, leading the formation of product with >90% yield. M.p. = 116–118 °C; $[\alpha]_D^{20}$ = 35.45° (*c* = 2, $CHCl_3$).



a) $KMnO_4$; b) $NH_2OH.HCl$, AcONa; c)$TiCl_3$;
d)LAH; e) $BF_3.Et_2O$, silicagel, toluene

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P4
21-HYDROXY-6β,19-EPOXYPREGN-4-ENE-3,20-DIONE

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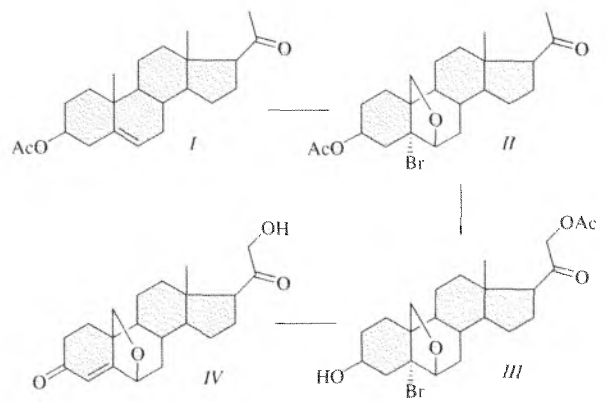
For diagnostic reasons we needed to synthesize 21-hydroxy-6β,19-epoxypregn-4-ene-3,20-dione (*IV*). The seven steps synthesis started with pregnenolone acetate (*I*). Though some steps of the synthesis have been described in literature[1-3], the complete synthesis from pregnenolone acetate has never been described.

Pregnenolone acetate (*I*) on addition of hypobromous acid afforded 5-bromo-3β-hydroxy-20-oxo-5α-pregnan-3β-yl acetate. The next step – in which the product was treated with lead tetraacetate and iodine under irradiation to obtain 6β,19-oxido-derivative (*II*) – was one of critical steps of the whole synthesis as for the yield. After saponification of 3β-acetate, the C-21 acetoxylation (lead tetraacetate, boron trifluoride etherate) was carried out to yield 5-bromo-3β-hydroxy-20-oxo-5α-pregnan-21-yl acetate (*III*). Oxidation with Jones reagent and dehydrobromination with sodium acetate followed. The last step, the hydrolysis with potassium hydrogencarbonate in methanol, was the second critical step. If this saponification is carried out under the presence of air oxygen, the yield is very low due to the side reaction to carboxylic acid derivative[4].



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P5
SYNTHESIS OF POTENTIAL METABOLITES OF BRASSINOSTEROIDS IN THE COCKROACH *PERIPLANETA AMERICANA*

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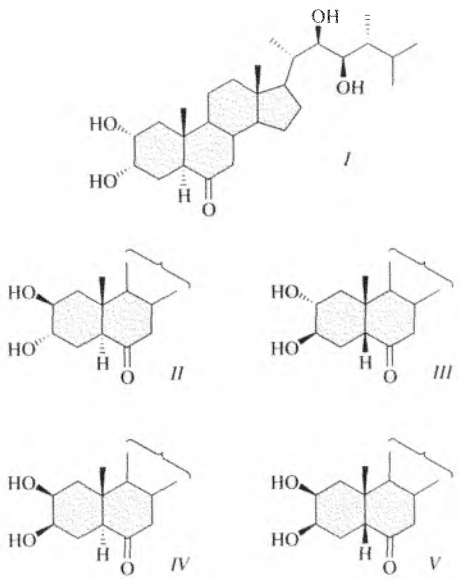
The brassinosteroids represent a new class of steroidal phytohormones of ubiquitous occurrence in the plant kingdom with high growth promoting and antistress activity[1].



Investigations of the metabolic conversion of the phytohormone 24-epicastasterone (*I*) in the cockroach, *Periplaneta americana* (L.) requires the availability of reference standards with structural elements of brassinosteroids and ecdysteroids. Therefore, some new compounds with 2,3-*cis* and *trans*-diol function, as well as A/B-*cis* and A/B-*trans* junctioned (*II–V*), were synthesized starting from ergosterol[2]. The structure-activity relationship will be discussed.

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P6
ALKALOIDS FROM THE POTATO AS STARTING MATERIAL FOR SYNTHESIZING STEROID HORMONES

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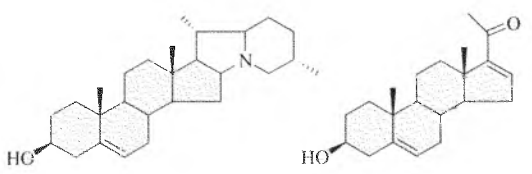
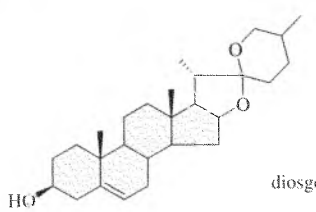
Large quantities of steroid alkaloids present in potatoes are produced in the starch industry. These glycoalkaloids are considered as waste products without any value, however after hydrolysis of the glycoside bond, a nitrogen-containing compound, solanidine *I*, is obtained which can serve as a starting material for synthesizing steroid hormones.

For solanidine to be used as starting material in the synthesis of steroid hormones, it has to be transformed into an 16-dehydropregnenolone *II*. This compound is presently obtained from diosgenine *III*.



solanidine *I* 16-dehydropregnenolone *II*



diosgenine *III*

Gasi *et al.*[1] have already shown that it is possible to transform solanidine into dehydropregnenolone but because of low yields and expensive reagents this method can't be applied in industry.

The current research will focus on the development of a new method in transforming solanidine into dehydropregnenolone which can be applied in industry.

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P7
THE TOTAL SYNTHESIS OF THE INSECT-ANTIFEEDANT DIHYDROCLERODIN

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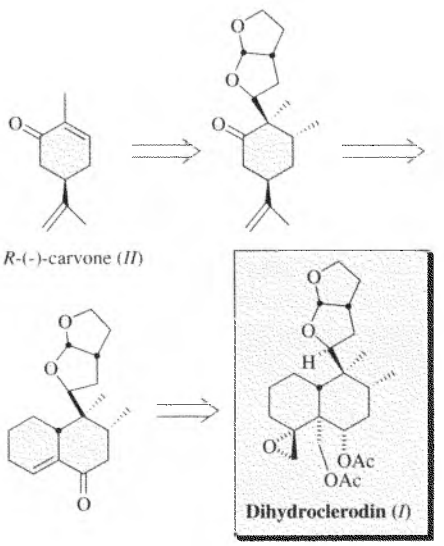
Clerodanes have a wide variety of biological activities. Dihydroclerodin (*I*) and similar clerodanes have been shown to be very potent antifeedants. The first total synthesis of dihydroclerodin (*I*) is developed starting from *R*-(–)-carvone (*II*) as homochiral starting material.



R-(-)-carvone (*II*)

In this total synthesis the hexahydrofurofuran, was introduced first[1] followed by the annulation of the second ring to construct the decaline system. In the last steps the two acetates and the epoxide in the lower part of the molecule were synthesised.

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P8
(+)-AROMADENDRENE AS CHIRAL STARTING MATERIAL FOR THE SYNTHESIS OF NATURAL PRODUCTS

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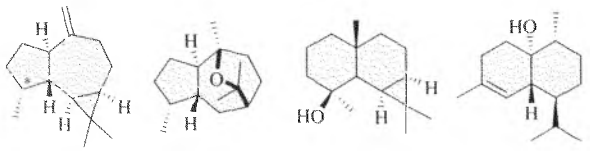
The tricyclic sesquiterpene (+)-aromadendrene is present in one of the distillation tails of *Eucalyptus globulus* in concentrations of 55–70%. It's five chiral centers and it's abundant availability make aromadendrene an attractive and cheap starting material for the synthesis of chiral products.

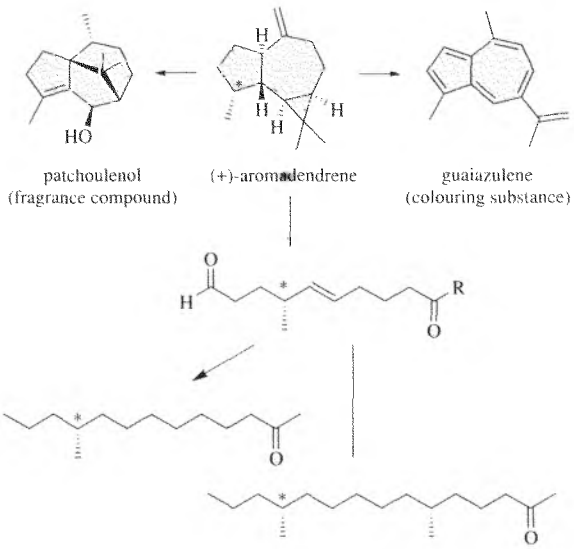
Previous research[1] on aromadendrene has led to the synthesis of various natural products, like (–)-kessane, (+)-maaliol and (–)-cubenol.



(+)-aromadendrene (–)-kessane (+)-maaliol (–)-cubenol

In the current research project, aromadendrene is used for the synthesis of natural products like flavours, fragrances and crop protecting agents. A few examples of possible products are patchoulenol, guaiazulene and sex pheromones from cucumber beetles.

The research that has been done on the synthesis of these pheromones will be presented on this poster.



patchoulenol (fragrance compound) (+)-aromadendrene guaiazulene (colouring substance)

(*R*)-10-methyl-2-tridecanone (sex pheromone of the southern corn rootworm)

(6*R*,12*R*)-6,12-dimethyl-2--pentadecanone (sex pheromone of the banded cucumber beetle)

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P9

OXIDIZED LABDANES AS STARTING MATERIAL IN ORGANIC SYNTHESIS

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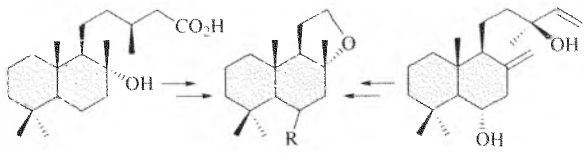
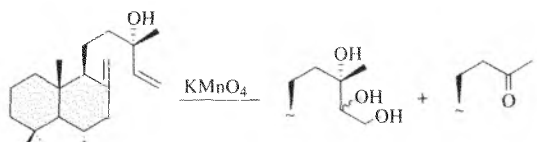
The gum of *Cistus ladaniferus* consists mainly of labdanolic acid. Larixol and in particular its 6-acetate (larixyl acetate) can be obtained as the major constituents from the oleoresin of *Larix europae*. It is of industrial importance to investigate possible uses for these labdanes as starting material in the synthesis of suitable target molecules. Degradation of the C_9 side-chain will lead to intermediates suitable for the synthesis of interesting terpenes like Ambrox®, a commercially important synthetic constituent of fine fragrances, and its C_6 derivatives.



labdanolic acid Ambrox and C_6 derivatives larixol

Several degradative labdane side-chain studies are known in literature but they do not appear to be of preparative value. Ogino[1] found that potassium permanganate solubilized in dichloromethane by use of a quarternary ammonium salt provides a convenient procedure for the oxidations of olefins. The degradative study of the side-chain of larixol and derivatives was performed using this reagent. Under proper conditions glycols or ketones could be obtained.



larixol glycol ketone

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P10
LARIXOL AS A BUILDING BLOCK IN TERPENE CHEMISTRY

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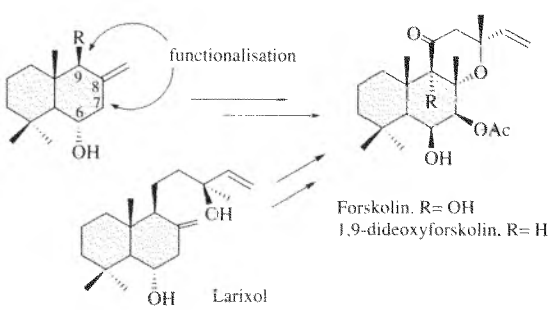
The oleoresine of larch (*Larix decidua*) contains large amounts of a diterpene named larixol *I* (>10% weight) which can be readily isolated in pure form. This renewable resource could be used for the synthesis of quite biologically active substances.

Indeed, viewing its chemical structure, larixol appears as a suitable starting material for the synthesis of drimanes and others terpenoids. The breakdown of the side chain of *I* to a functionalised one-carbon fragment leads to intermediates *II* which could be converted to bioactive drimanes.

Larixol *I* drimanes synthon *II*

Many diterpenes (drimanes and labdanes) have a very functionalised B-ring. An adequate functionalisation of its B-ring with hydroxy groups can be exemplified by the use of larixol as a starting material to get forskolin derivatives such as 1,9-dideoxyforskolin which shows good affinity for the protein GluT in its biochemical profile.



Forskolin. R= OH
1,9-dideoxyforskolin. R= H

Larixol

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P11
NEW APPROACH TO THE MARASMANE SKELETON *VIA* A TANDAM REARRANGEMENT CYCLOPROPANATION REACTION.

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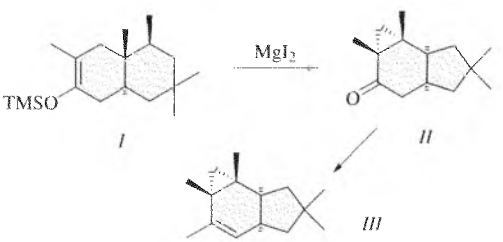
Marasmanes, belonging to the class of sesquiterpenes, are often found in nature as metabolite in fungi of genera *Lactarius* and *Russula*[1,2]. Many of these compounds possess interesting physiological activities like anti feedant activity. It is thought that these compounds take part in the chemical defence mechanism of the mushroom against predators, as they are formed, *via* an enzymatic conversion of a common precursor, when the mushroom is injured.

A new method for the construction of the marasmane skeleton is developed. The concept is based on the generation of a secondary cation via heterolytic cleavage of a mesylate group, induced by magnesium salts. This cation rearranges to a more stable tertiary cation which is intercepted by the nucleofilic double bond thereby creating the cyclopropane moiety *II*. The skeleton was completed by transformation of ketone *II via* its enol triflate into compound *III*.



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P12
ENANTIOSELECTIVE SYNTHESIS OF HIGHLY FUNCTIONALISED DECALONES STARTING FROM *R*-(–)-CARVONE

BEN J. M. JANSEN, TOMMI M. MEULEMANS, FLIUR Z. MACAEV, CINDY HENDRIKX, GERRIT A. STORK, and AEDE DE GROOT*

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The conjugate addition of dimethyl cuprate to cyclohexenones and trapping of the enolate as its trimethylsilyl enol ether, followed by a trityl perchlorate ($TrSbCl_6$) catalysed Mukaiyama-aldol reaction, was applied to *R*-(–)-carvone (see step 1, Scheme 1). This proved to be an efficient method for the preparation of C2,C3 functionalised chiral cyclohexanone synthons (see Fig. 1).

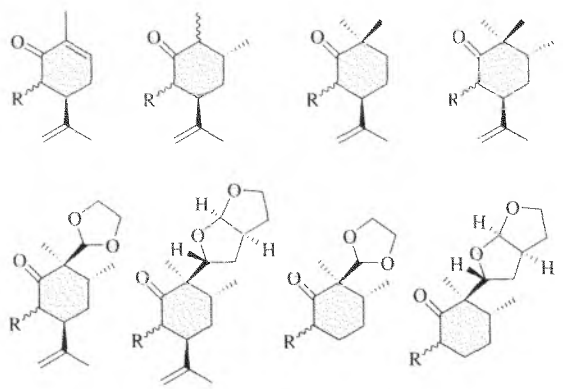
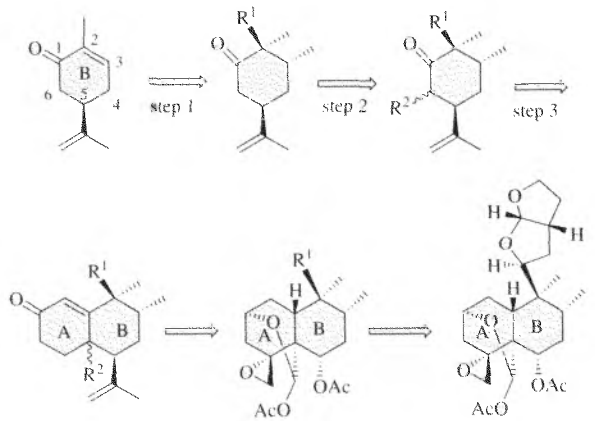


Fig. 1

These compounds were converted into their 6-cyano ketones (step 2, Scheme 1), which were submitted to Robinson type annulation reactions with methyl vinyl ketone (step 3, Scheme 1). The scope and limitations of this annulation was investigated. A series of highly functionalised chiral decalones were obtained that can be used as starting compounds in the total syntheses of enantiomerically pure clerodanes.



Scheme 1

P13
APPLICATION OF HIGH PLANT CELLS AND MICROORGAMISM CULTURES TO KETONE AND OXOLACTONE REDUCTION

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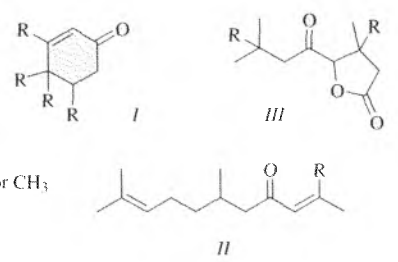
Synthesis of enantiopure compounds is nowadays an important issue due to the increasing demand for such molecules in pharmaceutical, agrochemical or food flavour industry. In this context the prochiral molecules containing carbonyl groups could be used as a substrates for enantioselective reduction.

In this paper we discuss the application of high plant cells and microorganism cultures to the reduction of oxolactones and α,β-unsaturated ketones. Potato (*Solanum tuberosum*)[1], artichoke (*Helianthus tuberosus*), apple (*Malus silvestris*) and six diffenrent microorganisms have been used as an biocatalysts of carbonyl group reduction.



Cyclic *I* and acyclic *II* α,β-unsaturated ketones were the first group of substrates which has been examined. Enantiomeric allylic alcohols obtained are applied in many organic syntheses.

The second group of substrates contained the δ-oxo-γ-lactones, that we had obtained and examined before. The bioreduction resulted in δ-hydroxylactones with ee = 70%.

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P14
STRUCTURE DETERMINATION AND FYSIOLOGICAL ACTIVITY OF A SESQUITERPENE HYDROCARBON IN *HELIOTHIS VIRESCENS*

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Receptor neuron responses to plant odours have been recorded in a species of heliothine moths, *Heliothis virescens*, by gas chromatography (GC) linked to electrophysiological recordings from single receptor neurons. Plant volatiles was collected by airation of intact and cut plant materials, including host and non-host plants of sunflower, tomatoe, chili peper, orange wild briar, spruce and juniper. Each neuron was tested for various plant volatile mixtures, both *via* a polar and a non-polar column. The two columns installed with a split at the end, lead half of the effluent to the GC-detector and the other half over the insect antenna. The chemical structure of the potent compounds were then determined by gas-chromatography linked to mass spectrometry (GC-MS). Most receptor neurons responded selectively to one or two components which were often present in several of the volatile mixtures. A large number (70–80%) of the neurons recorded from *H. virescens* showed selective response to the same component, appearing late in the gas chromatogram and shown by GC-MS to be a sesquiterpene hydrocarbon. This particular compound was earlier identified by GC-MS in *Piper cubeba* essential oil. Isolation and purification of the active compound was performed by using medium pressure liquid chromatography (MPLC) in two steps. Initially by separation of the hydrocarbons and the oxygenated compounds by using SiO_2 as adsorbent and hexane/ethylacetate as a gradient eluent. The hydrocarbon fractions were collected, followed by separation of the hydrocarbons using $AgNO_3$ (5 wt%) impregnated SiO_2. The chromatographic fractions were tested for neuron activity. One compound found in the fractions corresponded to the active sesquiterpene hydrocarbon found in the aeration samples by means of GC retention time, mass spectrum and neuron activity. The structure was determined by the NMR-techniques including ^1H and ^{13}C NMR, NOESY, HMQC, COSY and DEPT.

P15
OCCURRENCE OF PAIRS OF NEOCLERODANE C-12 EPIMERS IN *TEUCRIUM*

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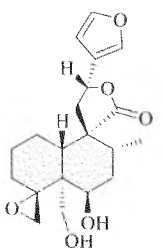
The genus *Teucrium* (Labiatae) is a rich source of neoclerodane diterpenoids. The main interest for these compounds arise from their antifeedant activity against pest insect.

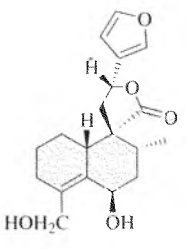
The occurrence of pairs of epimers at C-12 (*R versus S* configuration) was observed previously only once in *Teucrium kotschyanum* POECH, collected in the island of Cyprus, from which two pairs (*R* and *S*) were isolated. The *S* configuration is the more common in *Teucrium*, few products showing the *R* configuration.

We report on the investigation on *Teucrium maghrebinum* GREUTER et BURDET, collected in Algeria. The occurrence of six pairs of neoclerodanes epimers at C-12 is more unusual and seems to indicate a characteristic feature in the prochiral behaviour of some neoclerodane precursors. Of twelve products, six are new, the other six are already known as natural products:

- montanin B (12*S*) and 12-epi-montanin B (12*R*) (new)
- 19-deacetyl-teuscorodol (12*S*) and teusalvin C (12*R*)
- montanin D (12*S*) and 12-epi-montanin D (12*R*) (new)
- teucjaponin A (12*S*) and 12-epi-teucjaponin A (12*R*) (new)
- teukotshyn (12*S*) and 12-epi-teukotshyn (12*R*) (new)
- 19-deacetyl-18-acetyl-teuscorodol (12*S*) (new) and 12-epi-19-deacetyl-18-acetyl-teuscorodol (12*R*) (new)



12-epi-teucjaponin A (12*R*)



12-epi-montanin B (12*R*)

P16
EPALONS: B-HOMO DERIVATIVE SYNTHESIS

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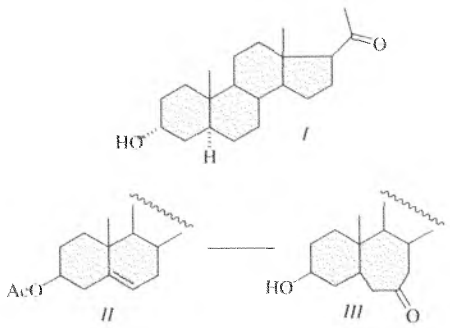
A potent and selective interaction of the steroidal anaesthetic alphaxolone with the $GABA_A$ receptor was demonstrated[1]. Subsequent studies established that certain naturally occurring steroids were potent positive allosteric modulators of the $GABA_A$ receptors. Although peripheral endocrine glands are an important endogenous source, the brain can synthesise "neurosteroids" and these have the potential to influence the activity of the $GABA_A$ receptor in CNS. Systemic administration of steroids have clear behavioural effects[1].

We prepared B-homo-7-oxo analogue *III* of the endogenous positive allosteric modulator $GABA_A$ receptors - 3α-hydroxy-5α-pregnan-20-one (*I*). Starting compound was pregnenolone acetate *II*. The target 3α-hydroxy-B-homo-5α-pregnane-7,20-dione (*III*) was produced in fourteen steps.



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P17
STEREOSELECTIVE SYNTHESIS OF 24-ALKYL-$\Delta^{22,25}$-STEROLS

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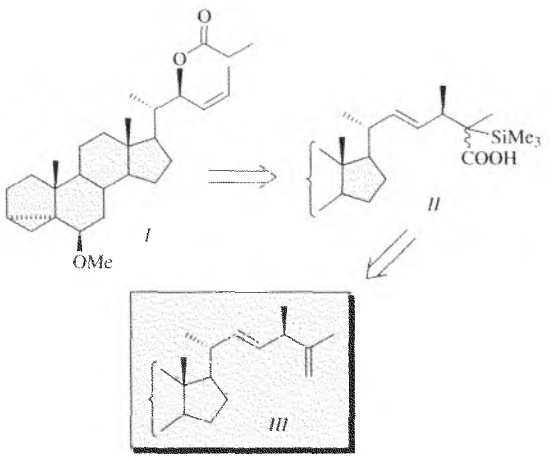
Elaboration of effective synthetic methods for preparation of Δ^{22}-24-alkyl derivatives is still a challenge[1]. Synthesis of related compounds containing an additional Δ^{25}-double bond characteristic of some marine sterols[2] is even more difficult task.



Here we want to report our approach to steroids with side chain *III* starting from easily available propyl ester *I*. The key step in the reaction sequence to the acid *II* was the Ireland–Claisen rearrangement. Introduction of Δ^{25}-double bond was based on the Peterson reaction.

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P18
1,3-DIPOLAR CYCLOADDITION OF NITRILE OXIDES WITH 17β-HYDROXY-17-VINYL STEROIDS

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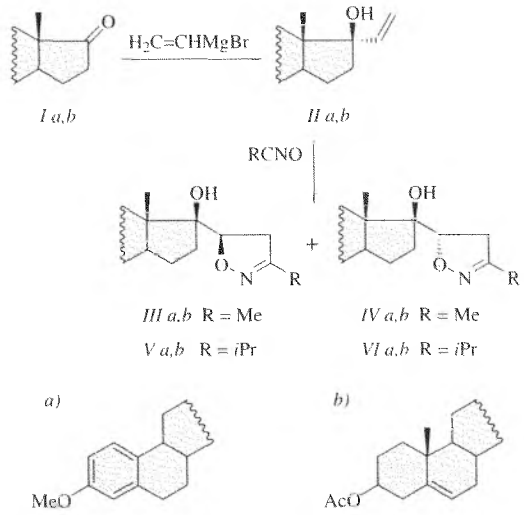
Selectivity of 1,3-dipolar cycloaddition of nitrile oxides with 17β-hydroxy-17-vinyl steroids *II* has been investigated. It was found that the reaction affords major epimer *IV* or *VI* which has *S* configuration of a new asymmetric centre at C-5′. The final stereochemical assignments have been done using X-ray analysis.



The diastereoselectivity of the cycloaddition depends on the steroid nucleus structure and reaches 95% in the case of 19-nor-steroids *VIa*. The details of the reaction and spectroscopic data of the synthesized compounds will be discussed.

The authors are grateful for the financial support from INTAS (grant INTAS 96-1109).

P19
APPROACH TO THE SYNTHESIS OF MODIFIED BRASSINOSTEROIDS

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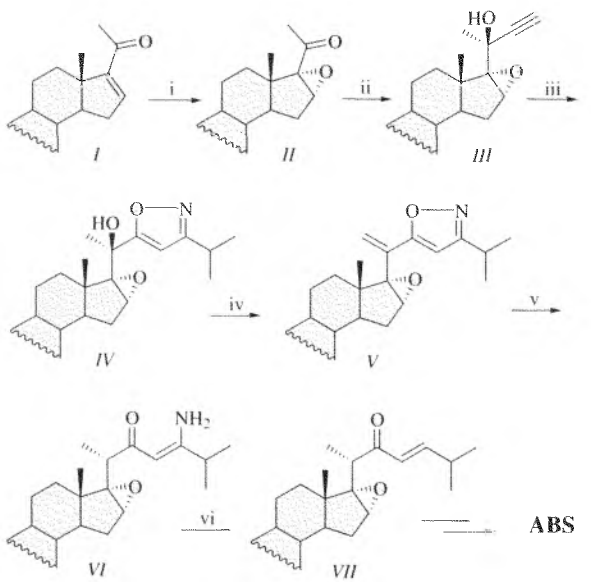
Because of our continuing interest to the synthesis of brassinosteroids (BS) and their functions in plants we have initiated a study towards synthesis of specifically modified brassinosteroids possessing all the characteristic functions of natural BS along with additional functional groups considering as possible sites for linking to a proteins for future immunochemical analysis.

Some of the BS analogues modified in D-cycle have been synthesized starting from 16-dehydropregnenolone acetate *I* *via* the 16α,17α-epoxides as shown in the scheme.



i) H_2O_2, NaOH, EtOH, 30-40 °C; ii) HCCMgBr, THF, 10-15 °C; iii) *i*PrCNO, Et_3N, $CHCl_3$, r.t.; iv) $SOCl_2$, Py, -40 °C; v) H_2/Ni-Ra, EtOH, r.t.; vi) Na/NH_3

The reaction conditions and the spectral data of the obtained compounds will be discussed.

The authors are grateful for the financial support from Fond of fundamental investigations of the Republic of Belarus (grant N x97-079).

P20
PREPARATION AND CYCLIZATION REACTIONS OF SOME 20,25-DISUBSTITUTED 22-ENAMINO-24-KETONES

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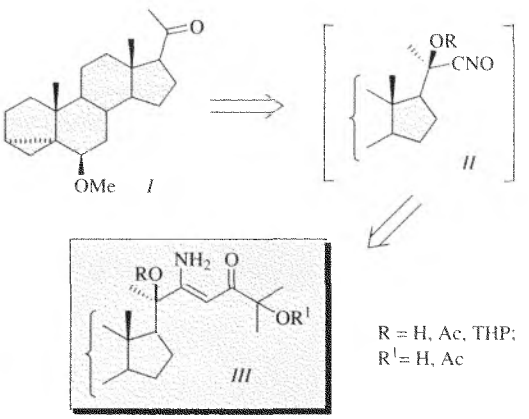
Steroidal 22C/23C nitrile oxides are useful intermediates for preparation of brassinosteroids[1–3] and vitamin D (ref.[4]) derivatives. Introduction of an additional functionality in α-position to the nitrile oxide group gives a possibility for preparation of new side chains, especially containing heterocycles. However, the presence of α-substituent makes the system in many respects different from simple nitrile oxides.



The present study deals with 1,3-dipolar cycloaddition reaction of 22C nitrile oxides *II* bearing a functional group (OH, OAc, OTHP) at C_{20} with 2-methyl-3-butyn-2-ol or its derivatives. The investigation of the cyclization reaction of the obtained enaminoketones *III* has been done as well.

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P21
SYNTHESIS OF SOME 5′-ALKYLFURAN-3′-ENE ESTRANE DERIVATIVES

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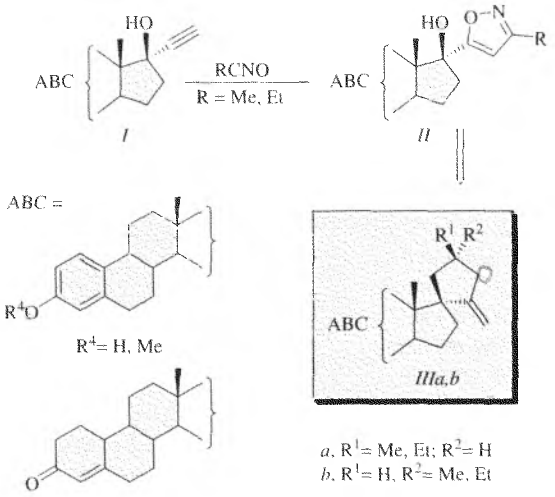
The 19-nor derivative of progesterone occupy a special place among pharmaceutically important steroids. A lot of compounds of this series have been registered as medicines and some other are under development[1]. Our efforts were directed towards synthesis of 19-nor steroids containing a tetrahydrofurane moiety with an alkyl group at the C-5′ position. The acetylenic alcohols *I* were used as starting materials. Their 1,3-dipolar cycloadditions with low-molecular nitrile oxides was used as the key step that led to the isoxazoles *II*. Further transformations ultimately led to the methylene derivatives *IIIa*, *IIIb*.



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P22
REARRANGED DITERPENOIDS FROM *SALVIA XALAPENSIS* BENTH. (LABIATAE)

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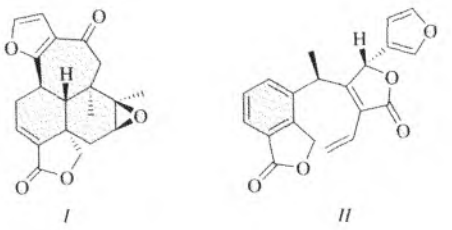
Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior de Cd. Universitaria, Coyoacán, 04510 Mexico, D.F.

The Labiatae (Lamiaceae) is a widespread and diversified family of plants with 224 genera and *ca* 4 000 species. The members of the family are found in the tropics, subtropics and temperate parts of the world. From the chemical point of view, the family have been subjected to intensive studies oriented to the isolation of several types of compounds. A large number of secondary metabolites such as sterols, flavonoids, iridoids, sesquiterpenes, diterpenes, triterpenes and sesterterpenes have been described from the roots and aerial parts of several members of the family. Some of them have shown interesting biological activities, such as, antispasmodic, antiviral, antibacterial, for example. Antifeedant activity against economically important insects has been observed for some neo-clerodane diterpenoids isolated from several members of the family. These plants are, therefore of medicinal and agrochemical interest[1].

Most of the 500 species of *Salvia* found in Mexico, Central and South America belong to the subgenus *Jungia* (formerly Calosphace). Several diterpenes have been isolated from this genus with abietane, neo-clerodane or rearranged neo-clerodane skeleton. Most of the diterpenoids isolated from the American *Salvia sp.* are neo-clerodane or can be biogenetically derived from a clerodane precursor. The languidulane skeleton, for instance, could be originated for the union of the C-16 to the C-1 of a clerodane and is somewhat distributed in the genus[2].



As a part of our ongoing chemical studies on Mexican *Salvia* species we report in this paper the structure of two new rearranged diterpenoids (*I–II*) isolated from the aerial parts of *Salvia xalapensis* BENTH (*Jungia*, Section Angulatae). While *I* possesses a languidulane skeleton, compound *II*, named salvixalapenolide, exhibited a new rearranged skeleton. We propose the name salvixalapenane to the new hydrocarbon skeleton of *II*, which could be considered a 5,6-seco salvigenane derivative. The structures of these compounds were established by spectroscopic means, including HMBC and HMQC spectra. A probable biogenetic hypothesis for the hydrocarbon skeleton of *II* is proposed.

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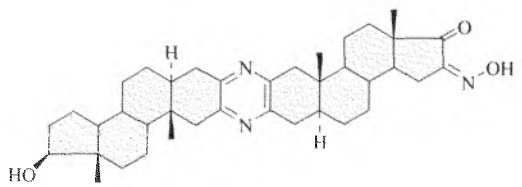
P23

STEROIDS AS BUILDING BLOCKS FOR THE SYNTHESIS OF RIGID AXIAL STRUCTURES

IVAN ČERNÝ, MILOŠ BUDĚŠÍNSKÝ, VLADIMÍR POUZAR, and PAVEL DRAŠAR

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

The studies of processes on biological membranes have shown the ability of rod-shaped polyols to form voltage dependent ion channels[1]. Various non-peptide structures were used, including polyphenylenes, *etc.* As an extension to these studies, new compounds based on the steroid skeletons bound together through pyrazine nuclei, as in cephalostatin[2], were proposed. The goal of building up the rod of the length about 40 Å can be achieved with about four steroid units.



Our initial studies covered methods for binding two steroid units both from side of A-ring and D-ring using symmetrical methods of coupling. Compounds based on pyrazine-A-bis-steroids are accessible by synthetic methods of cephalostatine analogues[3], using substitution of bromine in bromoketone with azide, its reduction to amine with simultaneous condensation, followed by air oxidation to pyrazine. Newly we prepared their pyrazine-D-bis-steroid counterparts, however in this case the yields were very low.

In the second stage we studied the possibility of further coupling. We found that the repeating of the synthetic route leading to bis-steroids is impossible due to reactivity of pyrazine present in the molecule. From the other routes, an approach based on the oximation with isoamyl nitrite turned to be the most promising. On the D-bis derivatives the reaction went smoothly, however in non-selective way giving predominantly bis-oxime product. On the A-bis derivatives, where only one position could be accessed, the reaction gave very poor yields, due to low solubility of the starting compound.

Support by grant of the Grant Agency of the Czech Republic #203/97/0695 is greatly acknowledged.

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P24

COUPLING OF STEROID *O*-(CARBOXYMETHYL)OXIME DERIVATIVES WITH MONO-PROTECTED α,ω-DIAMINOALKANES

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New approach to the preparation of steroid *O*-(substituted)oxime derivatives with the terminal amino group is presented.

The starting material were easily accessible steroid *O*-(carboxymethyl)oxime derivatives of type *I*. These derivatives were converted by the known procedure[1] to mixed anhydrides which reacts with BOC mono-protected α,ω-diaminoalkanes[2] to intermediates of type *II*. The BOC protecting group was cleaved with trifluoroacetic acid to afford desired compounds with terminal amino group of type *III*.

The reaction was tested with steroid derivatives with *O*-(carboxymethyl)oxime group in positions 7,17 and 19.

STEROID=N–OCH_2COOH *I*
STEROID=N–$OCH_2CONH–(CH_2)_n–NHCOOC(CH_3)_3$ *II*
STEROID=N–$OCH_2CONH–(CH_2)_n–NH_2$ *III*

In formulae *I–III*, $n = 2, 3$

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P25

CHEMICAL VARIATIONS IN THE ESSENTIAL OIL FROM *HELICHRYSUM ITALICUM* (ROTH.) G. DON

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Helichrysum italicum (ROTH.) G. DON is a shrub of the tribe Inuleae which belongs to the family of the Asteraceae. The plant emanate a characteristic odour, particularly the yellow-

ish flowers. Phytochemical studies of many species of *Helichrysum* have revealed that this genus produces α-pyrone derivatives, chalcones, flavones, di- and triterpenoids[1,2]. Some species of *Helichrysum* are known in folk medicine for their antimicrobial properties and antifungal activity[3].

In this study we report on the variations in the chemical composition of the essential oils of *Helichrysum italicum* obtained from plants collected from the same stand at different growth times. At the present we have identified in the oils, both from aerial parts and flowers, α-pinene, caryophyllene, α- and β-selinene, linalol and its esters, camphor as the main components.

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P26
DETERMINATION OF THE FUNGITOXICITY OF DIGITONIN

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Digitonin has been selected for determination of sensitivity of the method elaborated for testing fungitoxicity of chemical substances. Toxicity of digitonin was observed on *Fusarium culmorum* CCF 1839 (FC), *Fusarium solani* CCF 1333 (FS), *Cladosporium herbarum* CCF 1699 (CH), *Alternaria alternata* CCF 2672 (AA) and *Botrytis cinerea* CCF 2361 (BC). In our method, spores of microfungi were dissolved in 50 ml of sterile water. One ml of 2° liquid malt, 1 ml of solution of fungi and water solution of the tested substance were added into the test tubes. Digitonin with its well-known fungitoxicity served us as a model substance. The toxicity of digitonin and of selected pesticides (insecticide Actellic 50 EC with active ingredient (a. i.) pirimiphos-methyl, with fungicides Impact 125 EC with a. i. flutriafol, Tilt 250 EC with a. i. propiconazole, Tilt CB FW with a. i. carbendazim and propiconazole) were compared to verify the investigated method. Concentration of 25 g of digitonin on 1 ml of media manifested toxic effect on fungi. This metod demonstrated a higher sensitivity of the toxic effect on fungi comparing with the results described in papers[1]. Mycelium did not grow at AA, CH and BC. Only some hyphae grew at FS and FC, but they reminded on the bottom of test tube without continuing their growth. The effective concentration of digitonin was lower than the usual concentration of digitonin in seeds of *Digitalis* sp[2]. Toxicity of digitonin comparing with the toxicity of insecticide Actellic was significantly higher. Triazole fungicides that inhibite the biosynthesis of sterols were compared with digitonin known to form a specific complex with sterols. The negative interaction of these substances is thus expressed in the metabolism of sterols. The fungicide Impact showed a lower toxicity than digitonin. The toxicity of digitonine was in a close relations with toxicities of Tilt 250 and Tilt CB.

Supported by the Grant Agency of the Czech Republic, grant No. 203/98/0451.

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P27
THREE-DIMENSIONAL STRUCTURE-FUNCTION RELATIONSHIP OF VITAMIN D: FROM STUDIES OF CONFORMATIONALLY RESTRICTED VITAMIN D ANALOGS

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On the basis of conformational analysis of the side chain of 1,25-$(OH)_2D_3$ (*I*) and its 20-epimer (*II*), we grouped the side chain region in space of these vitamin D into four, A, G, EA and EG[2,3]. We then designed and synthesized analogs whose side chain mobility is restricted in one of these four regions. These analogs are four diastereomers at C-20 and -22 of 22-methyl-1,25-$(OH)_2D_3$ (*III–VI*)[1-4]. *In vitro* (affinity to VDR and DBP; gene transcription; cells differentiation) and *in vivo* (bone calcium mobilization and intestinal calcium absorption) activities of these analogs (*III–VI*) were evaluated. From the results we concluded that regions EA and A are important for VDR binding, gene transcription, cell differentiation and calcemic activity[5]. We similarly analyzed the conformation of the side chain of nearly fifty highly active known vitamin D analogs[6]. From the results a new space region termed F was found and our active space group concept was confirmed to be applicable to almost all these active vitamin D analogs. Altogether, the following space group-activity relationship was found: VDR affinity, EA>A> F>G>EG; DBP affinity, A only; Cell differentiation,

EA>F>A>EG and G; Calcemic, EA>A and G>F>EG. We modeled VDR-LBD using X-ray crystal structures of other members of nuclear receptor superfamily as template. Using VDR thus modeled, we are trying to develop our structure-function theory to that involving VDR.

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P28
A VERSATILE NEW SYNTHESIS OF RIBOSYLHOPANE AND BACTERIOHOPANEPOLYOLS

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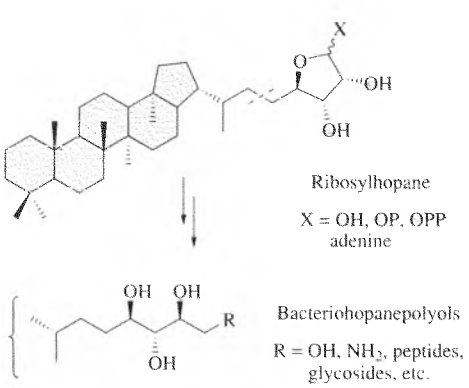
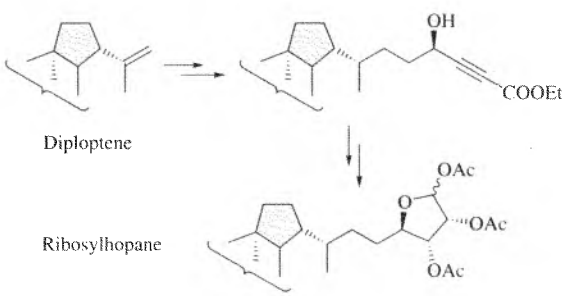
Bacteriohopanepolyols represent a particularly interesting class of triterpenoids[1]. They are widespread in bacteria and their role as membrane stabilisers in prokaryotes is generally accepted. Biosynthetic studies primary focused on the formation of the side-chain moiety more than ten years ago led to the surprising discovery of an alternative non-mevalonate pathway for isoprenoid biosynthesis which is now well documented. Several aspects concerning the side-chain biosynthesis, however, still remains largely obscure. Ribosylhopane was early proposed as a likely precursor for bacteriohopanepolyols and this hypothesis was supported by recent findings in bacteria of the corresponding lactone derivative[2].



Proposed biosynthetic formation of bacteriohopanepolyols from ribosylhopane

In order to further elucidate the biosynthesis of bacteriohopanepolyols, an efficient synthetic protocol for the preparation of ribosylhopane with good control over all asymmetric carbon atoms of the side-chain was developed. The synthesis is based on two chain elongations starting from diploptene by subsequent additions of two acetylenic moieties. In a key step a keto-propiolate is reduced stereoselectively to the corresponding hydroxy-propiolate by means of a chiral oxazaborolidine assisted hydroboration. This synthetic protocol represents furthermore a useful tool for the preparation of both natural and unnatural bacteriohopanepolyol analogues of biological interest.



Synthesis os ribosylhopane *via* an optically active propargyl alcohol

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P29
SEARCH FOR NEW NEUROSTEROIDS ACTING *VIA* $GABA_A$ RECEPTOR

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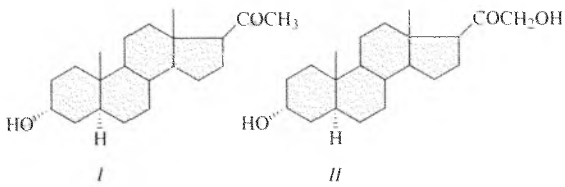
The present keen interest in neurosteroids[1] was launched when several metabolites of steroid sex hormones were found to be produced in the brain and for the brain: while the hormones are secreted by gonads and act *via* a slow process involving binding to a nuclear receptor and then to DNA which is followed by a genomic reaction, neurosteroids[2] (*e.g.* com-

pounds *I* and *II*) act almost immediately after affecting neuronal membrane receptors. In case of compounds modulating γ-aminobutyric acid receptors ($GABA_A$), their use as *antidepressants, anxiolytics, anticonvulsants* and analgesics is much restricted by their fast metabolism.



I *II*

We are trying to prepare new neurosteroids which would overcome their present drawback: their low stability and solubility in body liquids. Newly prepared compounds are screened *in vitro* (muscimol as standard) and promising substances also *in vivo*. A behavioural test (an intra species conflict test[1]) is used to show that some new compounds possess antiaggressive activity which is not achieved at the cost of the reduced readiness to flee or fight. Locomotion or social investigation is not affected by the compounds. For comparison, a prototype anxiolytic drug - diazepam - reduced not only aggressive behaviour of experimental mice but also the number of their defensive-escape acts.

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P30
SYNTHESIS OF 1α,25-DIHYDROXY VITAMIN D_3 ENANTIOMER

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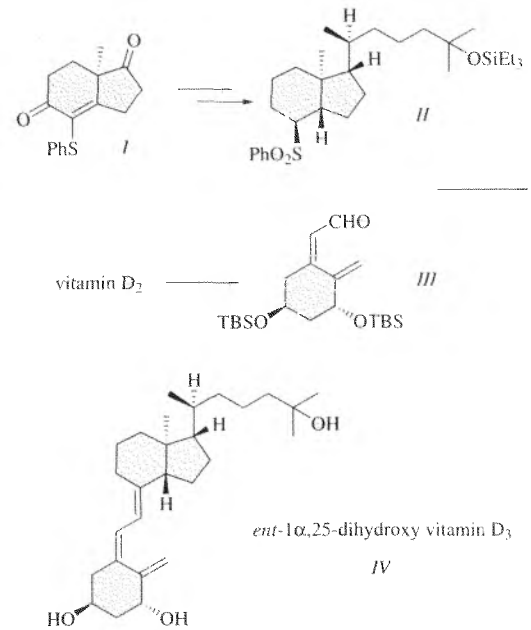
Analogues of 1α,25-dihydroxy vitamin D_3 with *iso*-configuration around asymmetric carbon atoms C-1, C-3, C-20 have received a great deal of attention[1]. Some analogues involving *ent*-ring A fragment have also been investigated[2]. We report the first synthesis of compound *IV* which is the enantiomer of natural 1α,25-dihydroxy vitamin D_3. The northern fragment building block *II* was prepared from dione *I* available by asymmetric annulation of 2-methyl-cyclopenta-1,3-dione with (phenylsulfanyl)methyl vinyl ketone[3]. The southern fragment *III* was obtained from vitamin D_2 applying selenium dioxide hydroxylation at the stage of its 7,8-dihydro-7,8-dihydroxy derivative[4]. Coupling of building blocks *II* and *III* was conducted according to the procedure of Kocienski *et al.* (ref.[5]).



ent-1α,25-dihydroxy vitamin D_3

IV

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P31
Cu-CATALYZED CONJUGATE ADDITION OF GRIGNARD REAGENTS TO ACTIVATED CYCLOPROPANES

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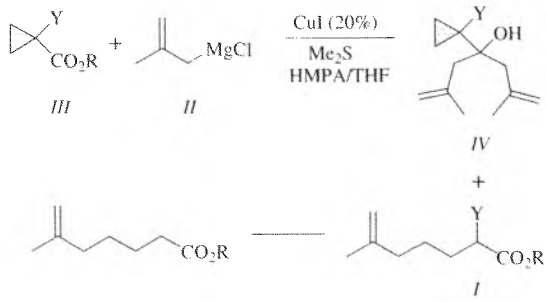
In course of studies on vitamin D total synthesis[1] ongoing in our laboratory we needed side-chain building blocks of general structure *I*. Preparation of some of these compounds

by conjugate 1,5-addition of a *iso*-butenyl lithium cuprate to 1,1-dicarboalkoxycyclopropane has been reported[2]. However, a large excess of the organolithium component was required in the described procedure and selectivity 1,5- *versus* 1,2-addition was rather low. We have found that the use of *iso*-butenyl magnesium chloride - cupric iodide reagent provides general economic approach to compounds *I*. Selectivity in reaction of *II* and *III* for various R and Y will be discussed.



Transformation of I, Y = CO_2Alkyl into I, Y = H by means of Krapcho decarboalkoxylation reaction and transformation of I, Y = SO_2Ph into I, Y = H by reduction with sodium amalgam will be also discussed.

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P32
Pd-CATALYSED ADDITION OF ORGANOZINC REAGENTS TO STERICALLY HINDERED VINYL IODIDES. SYNTHESIS OF CHOLESTEROL AND VITAMIN D ANALOGUES WITH AN AROMATIC RING AT C-17

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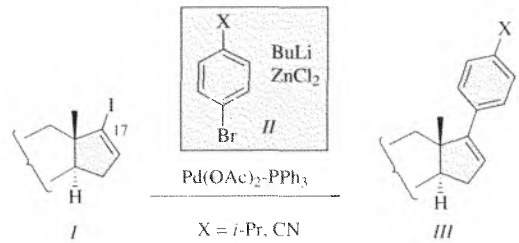
Vinylic iodides represented by structure *I*, easily accessible from the corresponding 17-oxo derivatives, are useful intermediates in steroid transformations. However, majority of reactions involving C–C bond formation on the expence of the C–I bond in *I* provide products with low yield due to very high sterical hinderance around C-17. We have found that palladium-catalyzed reaction of *I* and organozinc derivatives[1] generated from aryl halides *II via* lithio intermediates[2] affords coupling products *III* in excellent yields (80–85%). Synthesis of cholesterol and vitamin D analogues applying this reaction will be presented.



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P33
THE USE OF α-SULFONYL CARBANIONS IN CROSS COUPLING REACTIONS, APPLICATIONS IN NATURAL PRODUCT SYNTHESIS

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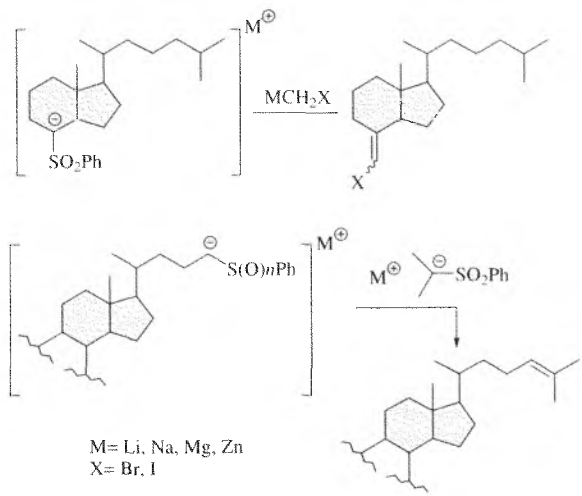
Cross-coupling reactions of α-metallated sulfones[1] and coupling reactions of α-metallated sulfones with other organometallics[2,3] provide a new and potentially useful approach to alkenylation. In this paper we present our results on the modifications of the steroid skeleton and/or side chain by the use of an anion anion cross coupling reactions as well as some model studies in that field.



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P34
SYNTHESIS OF A VITAMIN D - PREGNANE HYBRID ANALOGUE

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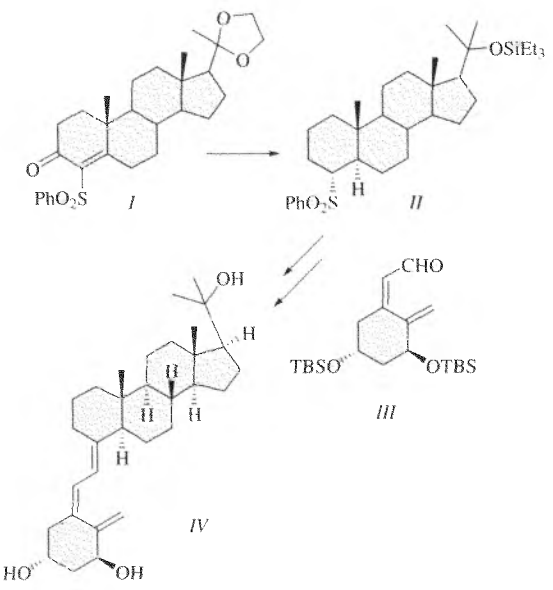
Discoveries of complex biological functions of 1α,25-dihydroxy vitamin D_3 (1,25-$(OH)_2D_3$) have stimulated interest in synthesis of variety of analogues of this hormone[1]. Recently, pregnane - based vitamin D compounds showing potentially useful biological properties were prepared[2]. We report a synthesis of vitamin D analogue *IV* which combines pregnane and vitamin D structures. The distance between 1α and 25 hydroxy groups in *IV* corresponds that in 1,25-



$(OH)_2D_3$ and the spacial arrangements well mimic the structure of the natural product, according to molecular modelling studies[3]. Vinylic sulfone *I*, easily accessible from progesterone, was transformed into its saturated 5α-derivative *II*. Coupling of *II* with ring A building block *III* followed by deprotection afforded the target compound.

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3. We thank Professor Sachiko Yamada for molecular modelling studies.

P35
SYNTHESIS AND BIOLOGICAL PROPERTIES OF *N*-ACYLPHENYLISOSERINATES OF SESQUITERPENOID ALCOHOLS OF *LACTARIUS* ORIGIN

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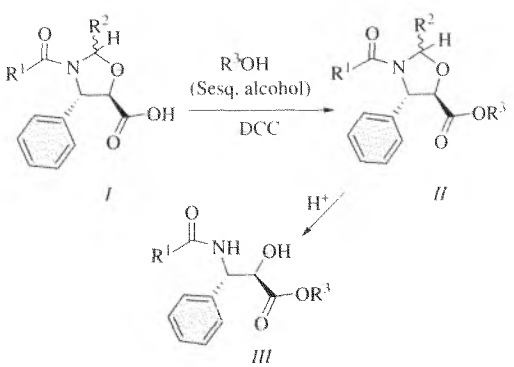
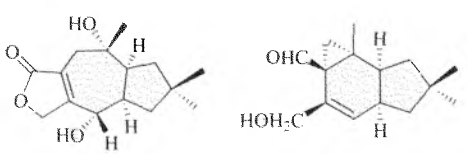
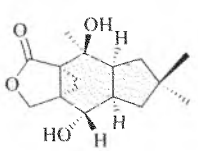
Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44, 01-224 Warsaw, Poland

Important biological properties of Taxol® *i.e.* 13-*N*-benzoyl phenylisoserinate (2*R*,3*S*) of baccatin *III* prompted us to synthesize and to check biological properties of various



The following sesquiterpenoic alcohols were used:





N-acyl phenylisoserinates of several sesquiterpenoid alcohols of *Lactarius* origin. Suitably protected *N*-acylphenylisoserine (*I*) when reacted with sesquiterpenoic alcohols in presence of

DCC gave appropriate esters (*II*). These, when hydrolyzed in acidic conditions produced *N*-acylphenylisoserinates (*III*). Biological properties of the compounds obtained are investigated.

P36
ROUTES TO VITAMIN E: PALLADIUM-MEDIATED ARYLATION OF OPTICALLY ACTIVE ACETYLENE AND ETHYLENE BUILDING BLOCKS

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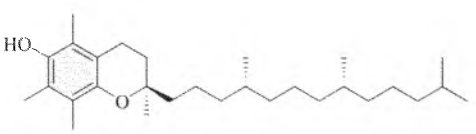
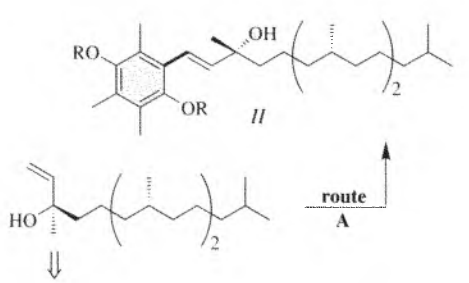
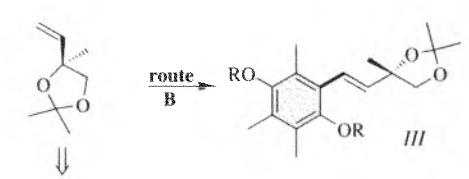
RRR-α-Tocopherol (*I*) exhibits the highest biological activity of vitamin E compounds and is, therefore, the most attractive target for total synthesis[1].

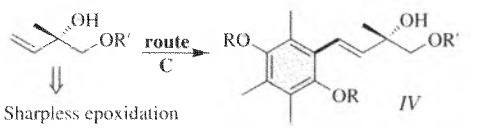


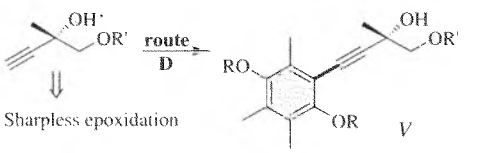
(2*R*,4'*R*,8'*R*)-α-Tocopherol *I*

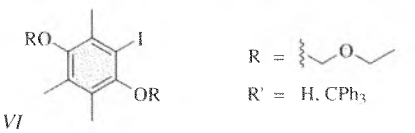
In a scheme to prepare this naturally occurring fat-soluble antioxidant, as well as homologues and derivatives thereof, we have applied Pd-catalyzed coupling reactions[2,3] to obtain chiral key-intermediates *II–V*. Protected iodohydroquinone *VI* has been used for arylation of functionalized, optically active isoprenoids, which have been obtained by chromatographic separation of diastereomeric camphanic esters (**route A**), enzymatic hydrolysis[4] (**route B**), or Sharpless epoxidation (**routes C and D**).











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P37
FINE CHEMICALS FROM TURPENTINE

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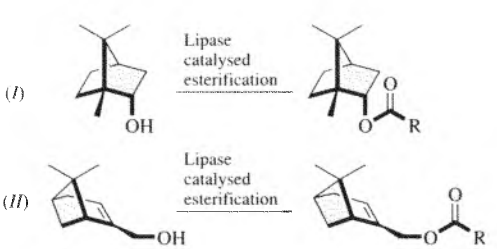
Today, more and more effort is put into the utilization of the natural resources in an optimal way. Thus, there is an increased interest in the exploitation of side products generated in different industrial processes. The high quality turpentine obtained as a side product from the thermomechanical pulping (TMP) process should constitute a source of valuable raw



materials for *e.g.* the flavour- and fragrance industry. The mild conditions during the TMP-process allows for a classification of the constituents of the turpentine as "natural".

The aim of this project is to find ways of transforming constituents of the turpentine into more valuable ones. In order to classify also the transformation products as "natural constituents" the methods for transforming turpentine constituents are limited to mild treatments, including enzymatic methods. Such methods have been used on the alcohol constituents of the turpentine, which can be isolated using distillation combined with chromatographic separation.

Lipases have been studied for esterification of the alcohols *via* transesterification. The substrate selectivity as well as the enantioselectivity of the enzymes have been determined. We have used borneol (*I*) and myrtenol (*II*) as model substances.

P38
THEORETICAL STUDIES AND MOLECULAR MODELING OF PREVITAMIN D PHOTOSYNTHESIS

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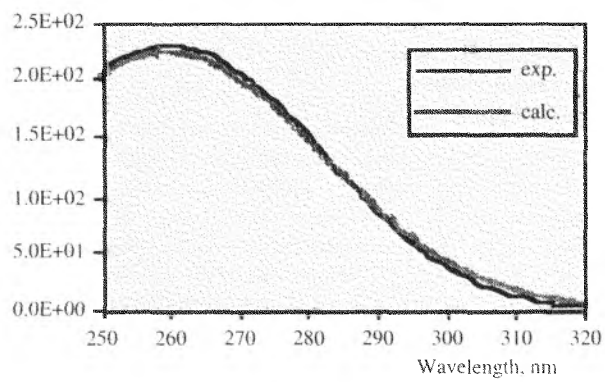
Due to the continued development of computing methods and capabilities, it has now become possible to model larger biomolecules using more sophisticated theocratical approaches. Such studies are aimed at gaining a deeper understanding of chemical and biological processes on a molecular level and probing structure function relationships.

The vitally important vitamin D synthesis is induced by natural UV-irradiation in the epidermis. This reaction sequence involves the photochemical ring-opening of the steroidal precursor 7-dehydrocholesterol to previtamin D and subsequent thermal rearrangement to the prohormone vitamin D. In contrast to the highly specific photoreaction occurring



in the skin, photosynthesis of previtamin D in solutions is complicated by a number of side-reactions. It has been found experimentally that the efficiencies of the photoreactions involved in the previtamin D photo-synthesis network depend highly on external factors like irradiation wavelength, reaction media (micro-environment) and temperature. Several theoretical models have been proposed to explain peculiarities of previtamin D photo-behaviour, but still there is a lack of a concise mechanistic picture. In this contribution, results of molecular modeling (forcefield calculation to density function studies) on previtamin D and its isomers will be disclosed and discussed in view of experimental observations and mechanistic models.

Our aim is to infer information about the predominant conformeres of previtamin D system through an interpretation of their electronic absorption spectra. For this reason it is necessary to model both the ground state and first excited states of each conformer studied. The preferred geometries of ground state previtamin D are the subject of debate.This complicates our understanding of the wavelength dependence of previtamin D photochemistry and the conformational effects on the reaction in general. We note the contributions to the observed spectra depend both on the thermal populations on the various ground state conformers and the oscillator strengths for transitions to the excited state. By combining the Boltzmann distributions obtained from ab initio calculations and density function studies with calculated excited state transition characteristics we obtain excellent agreement with the observed electronic spectrum of previtamin D.

O.D. acknowledges financial support through Fellowships from the Federal Ministry of Science of Austria and the U.S. National Science Foundation (CHE-9419102 to J. H. F.). Support from NSF to J. H. F. and Oesterreichische Nationalbank (Jubilaeumsfondsprojekt Nr.: 7395) to W. R. is gratefully acknowledged.

P39
A STUDY RELATED TO THE SYNTHESIS OF CEPHALOSTATINS

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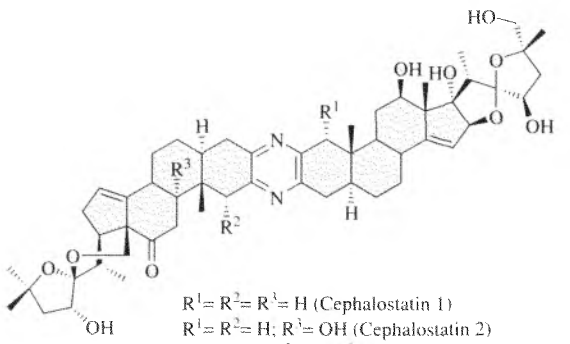
Cephalostatin have been isolated from the marine tube-worm *Cephalodiscus gilchristi* by Pettit's group[1] in 1988. These dimeric steroid pyrazines are among the most powerful anticancer agents with their subnanomolar antineoplastic activity. The most active member of the family are cephalostatin 1 (ref.[2]), its derivative hydroxylated at 9′α-position (cephalostatin 2), and the C-1 and C-1′ methoxy derivatives of cephalostatin 2 (cephalostatins 10 and 11, respectively).

We have elaborated two new methods of synthesis of the dimeric steroid-pyrazines. In one of them 2-nitro-3-oxo steroids are reduced with zinc in acetic acid followed by air oxi-

dation[3]. Another method consists in the direct reaction of 2-bromo-3-oxo steroids with ammonia. In the latter method a mixture is formed of the two C_2-symmetrical pyrazines, the "*trans*" and "*cis*" isomers. The isomers are easily separable



$R^1 = R^2 = R^3 = H$ (Cephalostatin 1)
$R^1 = R^2 = H$; $R^3 = OH$ (Cephalostatin 2)
$R^1 = OMe$; $R^2 = H$; $R^3 = OH$ (Cephalostatin 10)
$R^1 = H$; $R^2 = OMe$; $R^3 = OH$ (Cephalostatin 11)

by crystallization. The attempts of introducing of a methoxy group into a *quasi*-benzylic position in these systems have been undertaken. "*Trans*" and "*cis*" dicholestanopyrazines, along with their mono- and di-*N*-oxides, were subjected to oxidation under various conditions. The NBS/AIBN bromination at the *quasi*-benzylic position was more selective than oxidation. The 1α-bromo derivatives of "*trans*" and "*cis*" dicholestanopyrazines were obtained in addition to the minor 4α-bromo isomers. Acid methanolysis of the 1α-bromo steroids proceeded smoothly with retention of configuration at C-1. Molecular modeling studies have shown that 1α-methoxy derivatives are thermodynamic products.

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P40
A STUDY RELATED TO THE SYNTHESIS OF SAPONINS OSW

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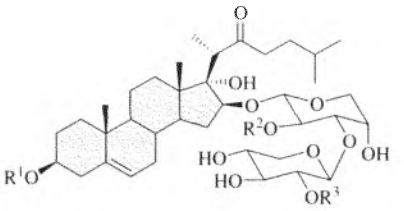
A family of cholestane glycosides isolated recently from *Ornithogalum sandersiae* by Sashida's group[1,2] shows an exceptionally strong anti-tumor activity. The cytotoxicity profile of saponin OSW-1 is strikingly similar to that of cephalostatins. The aglycon of OSW-1 is reminiscent of half of the cephalostatins, and therefore it is likely that they might have the same mechanism of action[3].



Saponins OSW:

R^1	R^2	R^3
H	Ac	4-methoxybenzoyl (OSW-1)
H	H	H
H	Ac	3,4-dimethoxybenzoyl
H	Ac	(*E*)-cinnamoyl
Glc	Ac	4-methoxybenzoyl
Glc	Ac	(*E*)-cinnamoyl

A study on the synthesis of both parts of saponins OSW[4], *i.e.* cholestane aglycone and disacharide moiety, has been undertaken. The starting pregnenolone was transformed into the 20-oxo-cholest-16-ene derivative. The α,β-unsaturated nitrile, obtained by the dehydration of the cyanohydrin of pregnenolone, was treated with isoamylmagnesium bromide to give the α,β-unsaturated ketone, which was isomerized to the β,γ-unsaturated ketone by treatment with a base. After protection of 20-oxo group as an ethylene glycol ketal the C_{16}–C_{17} double bond was oxidized with MCPBA to 16α,17α-epoxide. The similar epoxides were prepared from the steroidal Δ^{16}-22-ester and Δ^{16}-22-nitrile obtained from androstenolone upon reaction with phosphonoacetate or phosphonoacetonitrile, respectively, followed by the reaction with methylmagnesium iodide. A detailed investigation of the epoxide ring cleavage in these three systems, under basic or acidic conditions has been performed. A number of undesired side reactions, such as formation of the unsaturated compounds, lactones or hemiacetals, accompanied the epoxide ring cleavage. The steroid aglycone protected as 3α,5α-cyclo-6β-methoxy ether was glycosylated with the properly protected disaccharide trichloroacetimidate. The preparation of the β-D-xylopyranosyl-(1→3)-α-L-arabinopyranose derivative proceeded smothly using silyl groups for protection of hydroxyls and the trichloroacetimidate method for glycosylation.

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P41

A STEREODIRECTING EFFECT OF THE STEROIDAL 8(9)-DOUBLE BOND: SYNTHESIS OF THE 8(9)-DEHYDRO DERIVATIVE OF 13-EPI ESTRADIOL

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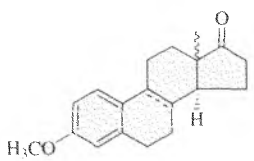
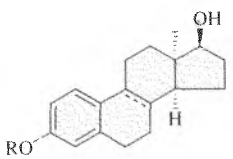
Estra-1,3,5(10),8-tetraene derivatives proved to be strong oxygen radical scavengers *in vitro* which displayed more or less feminizing effects[1]. Continuing our studies in this field, we set out to systematically invert selected chirality centers of the estra-1,3,5(10),8-tetraene core in order to gain additional information on the structure/activity relationship. We report here on the synthesis of 13-epi compound *V* from steroid *I* by 13-methyl group epimerization[2] to give tetraene *III* followed by 17-oxo group reduction (*IV*) and 3-methoxy group cleavage. When compared to the analogous sequence started with estrone methyl ether (*II*), both the epimerization of compound *I* and the 17-oxo group reduction of species *III* proved to be considerably influenced by the 8-double bond. GC/MS measurements were performed to quantitatively balance these reactions. Reduction of the 8-double bond in compound *IV* by use of the Birch protocol yielded 13-epi estradiol-3-methyl-ether (*VI*). This result showed that a *cis*-orientation of the C/D-rings caused by epimerization of the angular 13-methyl group does not change the "normal" steric course of 8-double bond reduction.





I: 13β; 8(9)-double bond
II: 13β; 8β-H, 9α-H
III: 13α; 8(9)-double bond

IV: R = CH_3; 8(9)-double bond
V: R = H; 8(9)-double bond
VI: R = CH_3; 8β-H, 9α-H

The steric consequence of the 8-double bond on the reduction of 13-epi 17-oxo steroid *III* will be discussed on the basis of X-ray structures.

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P42

STUDY OF COMPOSITION OF VOLATILE COMPOUNDS OF SIBERIAN MEDICAL PLANTS

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Medical and aromatic plants are of great importance as a source of useful ethereal oils. Composition of ethereal oils is not constant and depends on many things, including age of plants and their phase, and different ecological factors.

We have studied chemical composition of a number of species of the Asteraceae family growing wild in Siberia as well as the corresponding cultural forms from regional botanical gardens (*Artemisia dracunculus* L., *Artemisia obtusiloba* Ledeb., *Artemisia jacutica* DROB., *Artemisia abrotanum* L., *Artemisia scoparia* WALDST. et KIT., *Artemisia glauca* PALL. ex WILLD., *Artemisia altaiensis* KRASCH., *Artemisia glabella* KAR. et KIR., *Artemisia filatovae* A. KUPRIJANOV ssp. *nova*, *Artemisia pontica* L., *Brachanthemum baranovii* (KRASCH. ex POLJAK.) KRASCH., *Ajania fruticulosa* (LEDEB.) POJAK.) and 8 populations of *Thymus serpyllum* L.s.l. (Lamiaceae family) growing wild in Altai Mountains. Our data show that various factors, both endogenous and exogenous, can affect the composition of the essential oils of Siberian species of the above plants. Those species, which are characterised by significant polymorphism and broad natural habitats, usually demonstrate great dependence of the ethereal oil composition on geographical and climatic factors (*Thymus serpyllum*, *Artemisia dracunculus*, *Artemisia scoparia*, *Artemisia abrotanum*, and *Artemisia glauca*). Contrary, compositions of ethereal oils of endemic species, such as *Artemisia jacutica*, *Artemisia altaiensis*, *Artemisia obtusiloba*, do not vary significantly and demonstrate a hereditary predetermined set of secondary metabolites.

We studied also composition of hydrocarbons from oleoresins of *Abies alba* MILL., *Abies gracilis* KOM., *Abies mayranana* MIYABLE et KUDO, *Larix decidua* MILL., *Pinus funebris* KOM. and *Picea excelsa* LINK (*P. abies* KOR), as well as essential oils from shoots of *Abies sibirica* LEDB., *Pinus sibirica* R. MAYR. and *Picea obovata* L. of the population from Altai Mountains (Choiskii region). The data obtained allowed us to define more exactly composition of extracts and oils of conifers, which are of great importance as industrial source of wood and turpentine. We failed to observe a number of components that had been reported earlier to be constituents of the oils.

Compositions of volatile compounds were studies by GC-MS using quadruple MS (Hewlett–Packard MSD 5971) coupled to a HP 5890/II GC fitted with an HP-5 (30 m × 0.25 mm I.D., film thickness 0.25 μm) fused silica column. Quali-

tative analysis was based on comparison of the retention indexes and full mass spectra of the components with the data for standards prepared by separation of reference oils and identified by NMR spectroscopy.

Details of separation and identification procedures and chemical composition of the oils are discussed as well as dependence of the content of the principal components on different factors: age of plants and their phase, genetic and ecological factors.

The research was made possible in part by Grant "Study of volatile terpenoids of herbs from Siberia and Russian Far East" from The Competitive Centre on Natural Sciences at the Saint-Petersburg University.

P43

ISOPRENE CYCLODIMER IN A SYNTHESIS OF RACEMIC α-TOCOPHEROL AND (3'Z,7'Z/E,11)-α-TOCOTRIENOL

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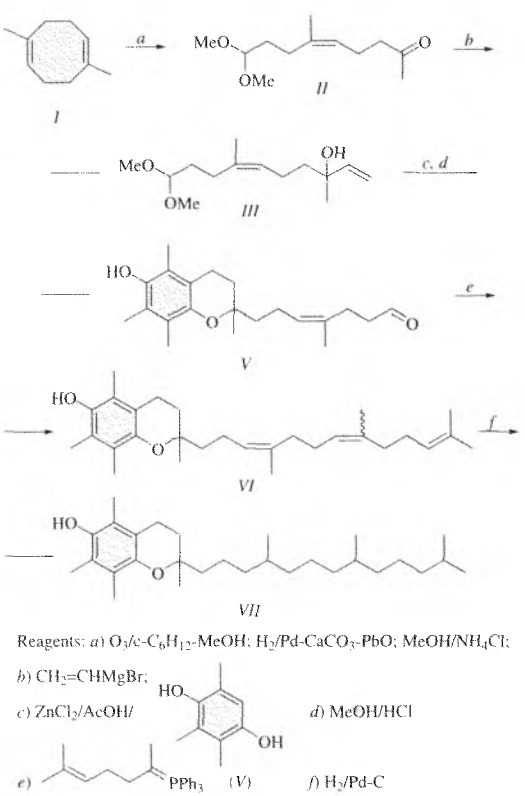
A partial ozonolysis of 1,5-dimethyl-1Z,5Z-cyclooctadiene (*I*) gives isoprenoid synthone *II* used in a synthesis of



α-tocopherol and its hexadehydroderivative - components of vitamin E. An interaction of ketoacetal *II* with vinylmagnesium bromide generates vinylcarbinol *III*, a condensation of the latter with trimethylhydroquinone and a subsequent hydrolysis gave chromane *IV*. An olefination of the latter by phosphorane *V* led to target α-tocotrienol *VI* and a subsequent hydrogenation gave racemic α-tocopherol *VII*.

P44

SYNTHESIS α-TOCOPHEROL ANALOGUES WITH A SIDE CHAIN FROM 6-METHYL-5-HEPTEN-2-ON AND (*S*)-3,7-DIMETHYL-6-OCTEN-2-ON

ANNA Yu. SPIVAK, VNIRA R. AKHMETOVA, NATALIYA V. LOBANOVA, GULNARA A. EMELIANOVA, and VIKTOR N. ODINOKOV

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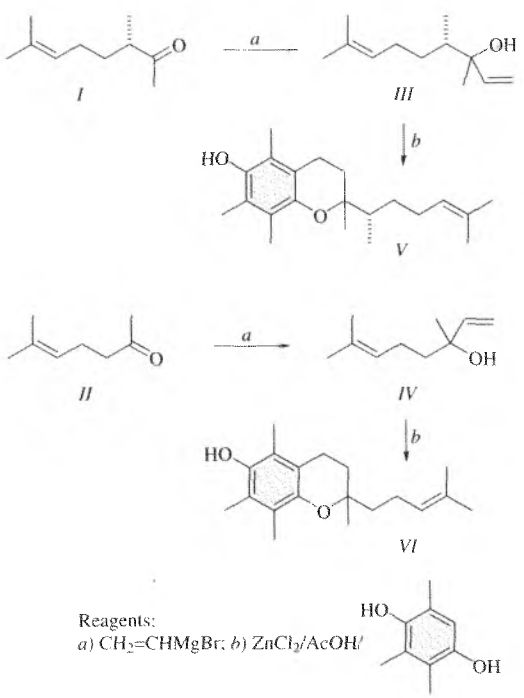
An interaction of terpene (*I*) and bis-*nor*-terpene (*II*) ketones with vinylmagnesium bromide gives corresponding vinylcarbinols *III* and *IV*, a condensation of the latters with



trimethylhydroquinone synthesizes 2-(6-methyl-5-hepten-2*S*-yl)-2*R*/*S*,5,7,8-tetramethyl-6-hydroxy (*V*) and 2-(4-methyl-3-pent-1-yl)-2,5,7,8-tetramethyl-6-hydroxy (*VI*) chromanes - analogues of α-tocopherols, isopropylidene group of a side chain of the latter is proposed to be used (for example, after

ozonolysis) for a synthesis of other analogues of α-tocopherol.

P45
20-HYDROXYECDYSONE AND ITS 22-ACETATE FROM *SERRATULA CORONATA*, ONE-STEP SYNTHESIS OF SHIDASTERONE 22*S*-ANALOG

ILGIZ V. GALIAUTDINOV, UMIRZAK A. BALTAYEV, and VIKTOR N. ODINOKOV

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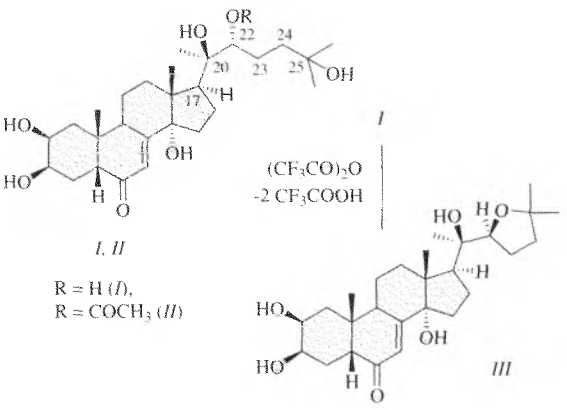
A novel method was developed to isolate ecdysteroids from a plant *Serratula coronata* (South Ural). A main component - 20-hydroxyecdysone (*I*) and its 22-acetate (*II*) was easily yielded (≥1.5%) from a sum of ecdysteroids. Shidasterone 22*S*-epimer was synthesised by the interaction of *I* and trifluoroacetic anhydride in chloroform.



P46
MASS-SPECTROMETRY OF RESONANCE ELECTRON CAPTURE - STRUCTURAL AND ANALYTICAL METHOD TO STUDY ECDYSTEROIDS

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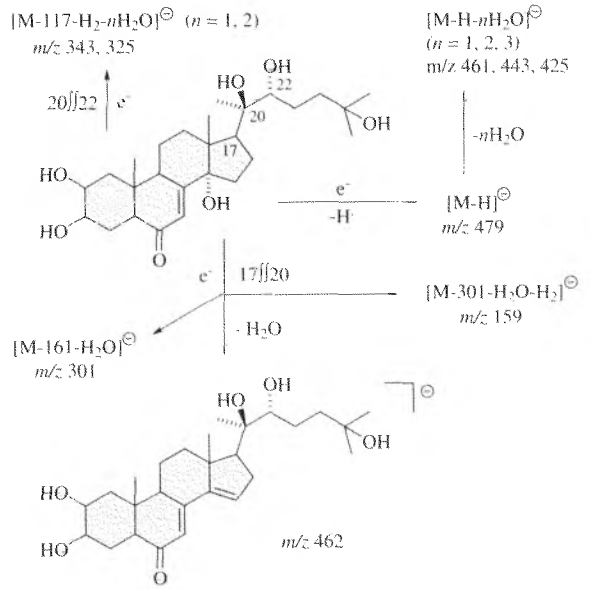
Mass-spectra (MS) are known to be an impotant characteristic to state a chemical structure of ecdysteroids. In contrast to mass-spectrometry of positive ions with electron impact, mass-spectrometry of negative ions (NI) in a mode of resonance electron capture (REC) is not widely used as structural and analytic method for investigation. On the example of 20-hydroxyecdysone we have shown an analytical importance of MS REC.

In contrast to MS of positive ions, a peak of molecular ion was not observed in NI MS of the styded object. However a pseudomolecular ion $[M - H]^-$ was generated in processes of REC. A further fragmentation is caused by dehydration and leads to ions $[M - H - nH_2O]^-$ (n = 1–3). A break of C(20)–C(22) bond in a molecule side chain gives ions $[M - 117 - H_2 - nH_2O]^-$ (n = 1,2) with m/z 325 and 343, positive ions m/z 327 and 345 are the analogs of the latters. A dissociation of C(17)–C(20) bond leads to negative ions m/z 301 and 159, characteristic ions m/z 300 and 161 correspond to the latters in positive ions MS. Ions $[M - nH_2O]^-$ (n = 1–3) and $[M - H_2 - nH_2O]^-$ (n = 0–3) together with similar positive ions $[M - nH_2O]^+$ are generated exclusively by a rearrangement mechanism. An analysis of electron structure for ion $[M - H_2O]^-$ (m/z 462) (with maximum intensity peak) shows the latter to be generated *via* a splitting of water molecule from a five-membered cycle (Scheme). A double bond conjugates with π-system to stabilize the lowest vacant orbital and to give a positive electron affinity of a molecule.



P47

SESQUITERPENES IN NATIVE ODOUR OF POTATO LEAVES *SOLANUM TUBEROSUM* L. AND COLORADO BEETLE *LEPTINOTARSA DECEMLINEATA* SAY

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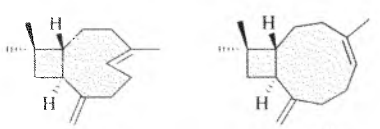
Native odour of plant leaves and flowers plays many important functions in a communication with insects. The paper describes results of identification of volatile chemical compounds secreted by potato leaves *Solanum tuberosum* L. and its pest - Colorado beetle *Leptinotarsa decemlineata* Say.

A concentrating of volatile organic compounds was carried out by dynamic gas extraction on adsorbent "Tenax GC" and "Carbotrap". A product separation proceeded on quarz capillary column, the product was analyzed by chromatomass-spectrometry method. Volatile secretions were identified *via* a comparison of experimental chromatographic characteristics with the known retaining indexes and by study of their mass spectra.

In result, sesquiterpene hydrocarbons in native odour of potato leaves *Solanum tuberosum* L. have been identified: *E*-caryophyllene (*I*), *Z*-caryophyllene (*II*), α-ilangene, β-elemene, Z-β-farnesene, aromadendrene, α-humulene, α-elemene, β-bisabolene, γ-cadinene, δ-cadinene.



I *II*

Compound *I* was observed in volatile secretions of Colorado beetle *Leptinotarsa decemlineata* SAY together with a minor component - germacrene-D in a ratio of 10 : 1. This fact evidences an active role of *E*-caryophyllene in the processes of interactions of a beetle with a plant - host. Olphactometric test showed a positive reaction of Colorado beetle to an action of *I* vapours. A dependence of potato eating on a main component content of native odour was stated by us, that evidenced its play as one of the components of food attractant for Colorado beetle.

P48

THE BIOSYNTHESIS OF PLANT TERPENES *VIA* THE NON MEVALONATE PATHWAY

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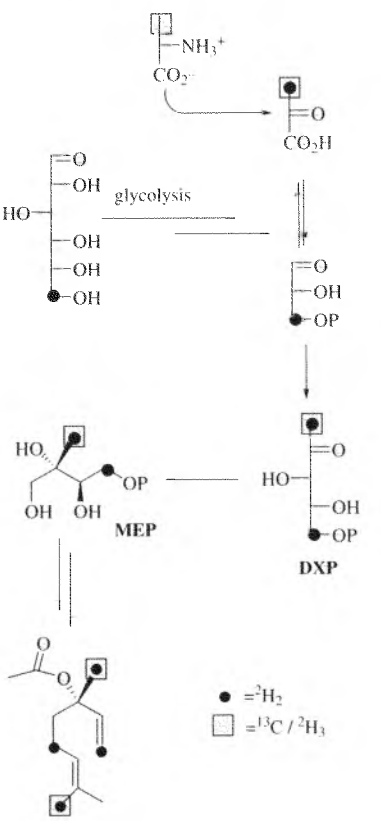
Recently[1] it has been shown that many plant and bacterial isoprenoids are biosynthesised thorough the C_5 sugar, 1-deoxy-D-xylulose-5-phosphate (DXP) rather than *via* mevalonic acid. An intermediate, 2*C*-methyl-D-erythritol phosphate (MEP) has been identified[2] in bacteria and the enzyme converting DXP to MEP overexpressed from *E. coli*[3]. The steps from MEP to isopentenyl pyrophosphate (IPP) are, however, unknown, and to this end we report incorporation studies of stable isotope (^{13}C, ^{2}H) glucoses and alanines to whole plant cultures of *Mentha citrata* demonstrating the conversion of MEP to IPP does not proceed *via* dehydrative steps.



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P49
4-SUBSTITUTED CANRENONE DERIVATIVES: PREPARATION AND BIOLOGICAL ACTIVITY

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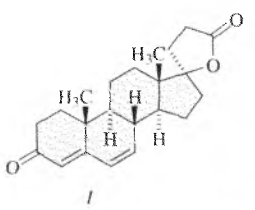
Canrenone (*I*) has been modified in order to increase its favourable but weak cardiac activity. A promising possibility to reach this aim seems to be the introduction of 2α-acetoxy or 2α-hydroxy groups because with uzarigenin (*II*) and its 3-*O*-acetate (*III*) this results in an remarkable increase in activity of the resulting compounds *IV* and *V* (ref.[1]). In order to get analogous canrenone derivatives we treated 3-oxo-5β,17α-pregnane-21,17-carbolactone[2] (*VI*, 100 mg; 0.29 mmol) in 10 ml dry benzene/acetic acid (1 : 1, v/v) with lead tetraacetate (194 mg; 0.44 mmol) and boron trifluoride etherate (0.22 ml; 1.75 mmol) as catalyst[3]. The mixture was stirred 4 hours at room temperature under nitrogen. Hydrolysis of the reaction products in methanol/10 % aqueous $KHCO_3$ solution (10 : 1, v/v) at room temperature overnight gave a crude product which was chromatographed on silica gel. Elution with



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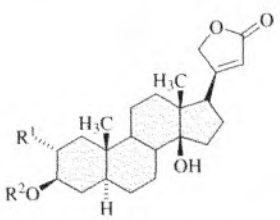
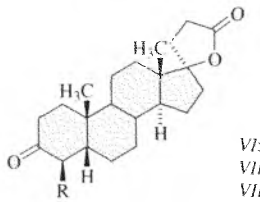


II: $R^1 = R^2 = H$
III: $R^1 = H$; $R^2 = COCH_3$
IV: $R^1 = OH$; $R^2 = H$
V: $R^1 = OCOCH_3$; $R^2 = H$



VI: R = H
VII: R = OH
VIII: R = $OCOCH_3$

hexane/chloroform (3 : 2) gave 4β-hydroxy-3-oxo-5β,17α-pregnane-21,17-carbolactone *VII* (46 mg; 44 %) which crystallized from chloroform/hexane as colorless leaflets (m.p. 203–206 °C). TLC on silica gel 60 F_{254} Merck (chloroform; 3×): $R_F = 0.31$. EI-MS: *m/z* 360.4 ($C_{22}H_{32}O_4$, M^+). Acetylation of *VII* with acetic anhydride/pyridine gave *VIII*. Needles from chloroform/hexane (m.p. 228–231 °C). EI-MS: *m/z* 402.1 ($C_{24}H_{34}O_5$, M^+), 360.1 ($M - CH_2{=}C{=}O$), 342.1 ($M - CH_3COOH$). The position and stereochemistry of the equatorial 4β-*O*-acetyl group has been derived from the ^{1}H-NMR spectrum which shows a doublet at δ 5.52 ppm (^{1}H, d, $J_{4\alpha,5\beta} = 12.2$ Hz; 4α-H). Contrary to the 3-keto-5α-steroids which react with lead tetraacetate under formation of 2α-*O*-acetates[3], 3-keto-5β-steroids are acetoxylated in the 4β-position. The biological activity of the compounds was determined in the ATPase test using Na^+/K^+-transporting ATPase from human kidney[4] or human heart[2]. *I*, *VI*, *VII* and *VIII* show about the same activity and nearly no differences in the kinetic constants[2-4]. The introduction of 4β-*O*-acetyl or 4β-OH in 5β-steroids does not improve the biological activity.

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P50
ASSIGNMENT OF STEREOCHEMISTRY TO CHLOROFLUOROACETIC ACID ESTERS OF CHIRAL SECONDARY ALCOHOLS

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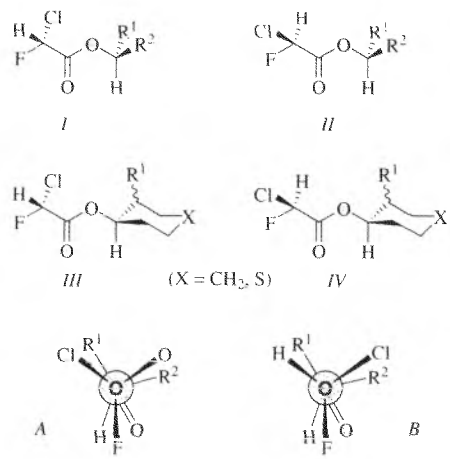
An appealing method for determining of particular enantiomers is the direct separation using chromatographic techniques either on chiral stationary phase or with chiral solvating agents. However, it will be hardly found such a phase and/or solvating agent which would be suitable for analyzing of structurally different chiral compounds so it seems that the investigation of enantiomers by means of derivatization in a non-chiral environment still makes sense. In this context, we turned our attention to (*S*)- and (*RS*)-chlorofluoroacetic acid (CFA) which we have already proven to be a valuable tool in an alcohol chiral analysis: (1) CFA with alcohols affords diastereoisomers relatively easily even when other means of derivatization fails and (2) the chromatographic behavior of CFA esters appears to be superior over those obtained by *e.g.* Mosher's procedure. According to the ^{1}H NMR data and quantum-chemical calculations, CFA diastereoisomers fit the Mosher's and Helmchen's ester-stereomodel very well with apparent large energy difference among particular conformers (**A** and **B** seem to be the most stable ones). In order to find out the scope and limitations of this

principle in stereochemical analysis, more than twenty diastereoisomeric pairs of common structures *I–IV* have been prepared and the stereochemistry of esters obtained was correlated with their ^{1}H NMR, HPLC and GC data. The HPLC



and GC data of particular diastereoisomers show the periodicity of chromatography behavior. These results indicate possible use of CFA derivatization in assigning stereochemistry of chiral secondary alcohols.

The authors acknowledge financial support by the Grant Agency of the Czech Republic, Grants No. 203/97/0037 and 203/98/0462.

P51
A NEW ACCESS TO 2-ALKYL SUBSTITUTED SECONDARY ALCOHOLS. APPLICATION TO THE SYNTHESIS OF 4-METHYLHEPTAN-3-OL

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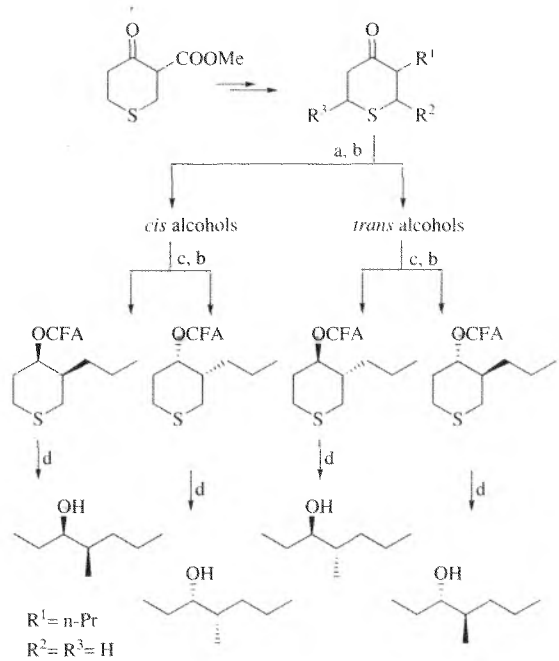
2-Alkyl substituted secondary alcohols represent a common structural feature for a many of biologically important natural products. Among them, pheromones with this environment attracted considerable attention due to their potential use as a modern insecticides. Searching for a simple and efficient method for preparing of some those compounds with defined stereochemistry, we turned our attention to the substituted 4-thianols whose synthetic utility has already been documented. Our present poster demonstrates a model study leading to all four stereoisomers of 4-methylheptan-3-ol, *i.e.* insect pheromone component of considerable importance. The title compounds were prepared by five-step route starting from 4-thianone (R_1 = n-Pr; R_2, R_3 = H). Key steps in the synthesis include (i) reduction of 3-propyl-4-thianone to yield an



a) $LiAlH_4$; b) SiO_2 separation; c) (*S*)-CFA, DCC; d) RaneyNi/CH_3OH

easily separable isomeric mixture of *cis*- and *trans* 3-propyl-4-thianols, and (ii) highly efficient resolution of the particular *cis*/*trans* isomers by means of a chromatographic separation of their respective esters with (*S*)-chlorofluoroacetic acid ((*S*)-CFA). Subsequent hydrolysis and desulfurization afforded finally, all four stereoisomers of 4-methylheptan-3-ol in about 18% overall yield and purities better then 90%.

The authors acknowledge financial support by the Grant Agency of the Czech republic, Grants No. 203/97/0037 and 203/98/0462.

P52
BRASSINOSTEROIDS AS PERSPECTIVE PLANT GROWTH REGULATORS IN THE CONTROL OF FIELD CROPS

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Brassinosteroids can control growth of the field crops if applied in 10–100 mg[1]. We examined the possibilities of using 24-epibrassinolide (24-epi) and the 4154-brassinosteroid (4154-BR)[2] in controlling the yields. These processes can be of perspective in agriculture by influencing some growth

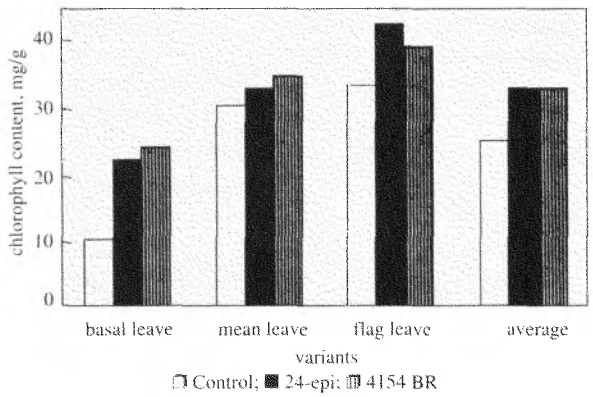

Fig. 1. Chlorophyll content in a vertical transect by stands of SANDRA wheat and changes after the treatment by brassinosteroides

characteristics directly related to farming grain yield (DM accumulation, chlorophyll content, leave area, a. o.) and the activity of enzyme glutamate kinase (GK; GK catalyzes the biosynthesis of proline which plays an important role in plants

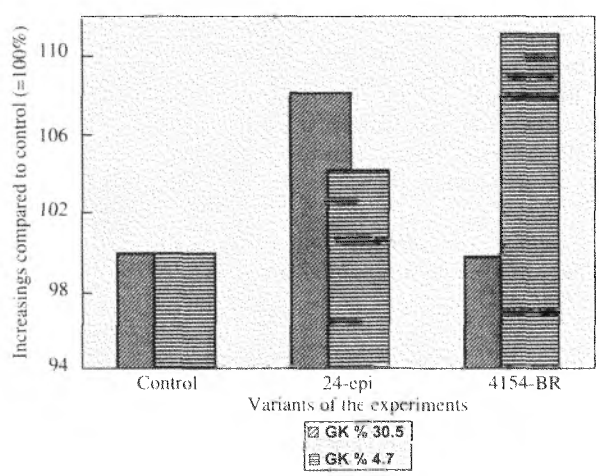

Fig. 2. Activity of the enzyme glutamatekinase in flag leaves of wheat SANDRA treated by brassinosteroides

especially in relation to overcoming stress caused *e.g.* by temperature, drought, salinity, and also during the ontogenesis[3–5]).

Experiments were carried out on a model of wheat SANDRA (400 grains/m^2 in n = 4 repetitions with 10 m^2/plot). 4154-BR and 24-epi were applied as a spray with an aqueous solution, $c = 1$ mol·10^{-6} + 0.05 % V/V of surfactant Cyttowet. In regular intervals leave area, DM per plant, number of tillers (fertile and sterile ones), flowers and grains in a ear were tested by IBP methods[6]. After the harvest the yield (t/ha), the length of ear, and 1000 grain weight were used as criteria. The chlorophyll content in an acetone extract was estimated spectrophotometrically. The activity of enzyme was determined by a modification of the hydroxamate method[7].

There are differences in activity of 24-epi and 4154-BR in ear elongation (24-epi: 157.2 ± 4%, 4154-BR: 151.9 ± 7.8%) but not in stem (116.3% and 117.8%). The control in all cases = 100 %. The substances enhance tillering (24-epi: 154.9 ± 9.3%, 4154-BR: 157.8 ± 9.6%), assimilative area LAI and LAD (24-epi: 133.7%, 4154-BR: 136.8%). The number of grains was also increased (control: 31.5 ± 2.6 grains, 24-epi: 41 ± 3.2, 4154-BR: 42.5 ± 5.7) as well as their weight (control: 100%, 24-epi: 110.6 and 4154-BR: 109.3%). Chlorophyll content (Fig. 1) in treated plants was significantly higher than in control plants. 24-epi enhanced the activity of GK to 108.1% of control but 4154-BR declined it to 99.72% of control (Fig. 2). Grain yield reached 4.75 t/ha in control, 6.12 t/ha after 24-epi and 6.03 t/ha after 4154-BR application.

This work was supported by the Grant Czech Univ. of Agriculture No. 2060/10/18697/0.

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P53
DESIGN AND PREPARATION OF IMMOBILISED BRASSINOSTEROIDS FOR ISOLATION OF BRASSINOSTEROID RECEPTORS

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Brassinosteroids (BRST) are a group of plant steroids eliciting remarkable growth responses. They are biologically active in the various bioassay systems designed for gibberellins, auxins and cytokinins. The molecular and biochemical analysis of *Arabidopsis* mutants has furnished conclusive evidence that BRST are plant growth hormones[1]. The molecular mechanism of BRST action is uncertain, although one might argue from structural considerations that they are likely to work by a mechanism similar to that of animal steroid hormones which generally act *via* a soluble receptor-ligand complex that binds to nuclear sites to regulate the expression of specific genes. Despite many studies on plant steroids there is no report on successful isolation of a receptor.

Our aim was to prepare affinity chromatography carrier for protein receptor isolation from plant extracts (with special respect to *Arabidopsis thaliana*) through BRST analogues bound covalently by a proper spacer arm. The ligand must be bound by that part of the molecule which least participates in the biospecific binding. For this reason oriented immobilisation of the ligand to the matrix was necessary. First attempts were through carboxyl group introduced into the side chain. Successful binding to MBHA carrier yielded 0.08 mg of steroid per gram of matrix. The use of *N*-succinimide did not give satisfactory results. GABA proved to be a satisfactory spacer arm for this purpose. Good yield gave agarose modified by adipic acid dihydrazide, too. The amino groups of the polyacrylamide-based carrier PEGA were occupied by BRST with 85% yield.

Among others newly synthesised BRSTs (20*S*)-2α, 3α-dihydroxy-7-oxa-B-homo-5α-pregnan-6-on-20-carboxylic acid was used for immobilisation. This compound was obtained in eight steps by general synthesis of brassinosteroid skeleton[2,3] from bisnorcholanic acid, *i.e.* from 3β-hydroxy-23,24-dinor-5-cholenic acid.

The plant extracts were obtained by grinding frozen plant leaves, salts were removed by gel filtration and the extract applied to the bioaffinity matrix. The proteins bound by non-specific sorption were eluted by the same buffer with increasing gradient of NaCl. After 2 N NaCl also acetic acid was used for elution. All samples were desalted on PD 10 columns, concentrated by lyophilisation and used for electrophoretic estimation of the proteins present.

The work was sponsored by grant A4055803 of the Grant Agency of the Czech Academy of Sciences and the German Federal Ministry of Education and Research, German-Czech co-operation project TSR-043-97 (ME-081).

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P54

THE SYNTHESIS OF PTERULONE – AN INHIBITOR OF ATP-SYNTHESIS IN FUNGI

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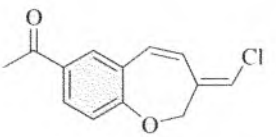
The fungal metabolite pterulone was isolated from feromontations of a *Pterula* species and was shown to exhibit strong antifungal but weak cytotoxic activities. The antibiotic is an inhibitor of eucaryotic respiration, interfering with the NADH:ubiquinone oxidoreductase[1]. It was isolated in small amounts from the mycelium of the fungus and inhibited the respiration rate of bovine heart mitochondria using NADH as substrate with an IC_{50} value of 36 μM.



We report the synthesis of pterulone and some derivatives and analogues. In addition, structure-activity relationships will be discussed.

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P55

ANALOGUES OF THE SESQUITERPENE ISOVELLERAL: PROBING THE STRUCTURAL REQUIREMENTS FOR BIOACTIVITY

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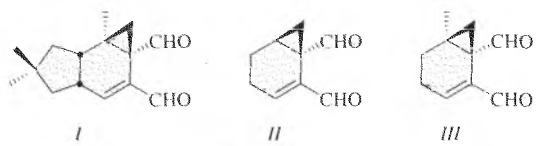
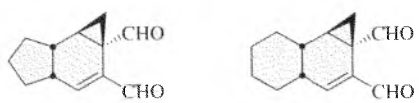
Many naturally occurring terpenes contain an unsaturated 1,4-dialdehyde functionality. Unsaturated dialdehydes are often biologically active[1] possessing for example antifeedant, antifungal, mutagenic cytotoxic and antimicrobial activities. The activities are due to the reactivity of the unsaturated 1,4-dialdehyde functionality but even small structural changes and/or isomerisation of an active compound can greatly affect and modulate the activity[2]. One of the most potent members of this class is the sesquiterpene isovelleral[3] (*1*), isolated from the fruitbodies of the basidiomycete *Lactarius vellereus*.

In an ongoing study of structure-activity relationships for isovelleral (*I*) it has been shown that that the monocyclic analogue *II* lacking the methyl group next to the cyclopropane



I *II* *III*

ring has enhanced activity[4], probably due to the removal of steric hindrance making the molecule more susceptible to nucleophilic attack, while analogue *III* is less active. Presumably, the second ring is needed in order to reduce the flexibility of the first ring and give the correct conformation of the aldehyde groups.



IV *V*

Our conclusion is the isovelleral analogue (*IV*) lacking the three methyl groups would be very potent and is was therefore synthesized. A six membered ring analogue (*V*) was also prepared in order to obtain further structure-activity relationships.

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P56
MINOR ECDYSTEROIDS FROM *LEUZEA CARTHAMOIDES*

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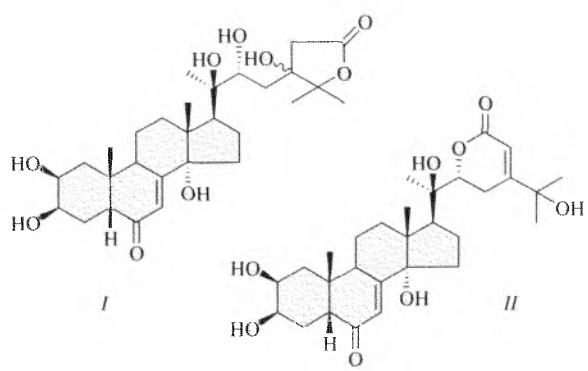
Leuzea carthamoides (syn. *Rhaponticum carthamoides*), species endemic in Siberia but cultivated in a large scale as a medicinal plant in Europe, is known to contain various ecdysteroids, insect moulting hormone analogues. Roots of this plant have been used in our laboratory as a most convenient and rich source of basic phytoecdysones, *e.g.* 20-hydroxyecdysone, polypodine B, ajugasterone C, makisterone A and some of their mono- or di-acetonides[1]. There were utilised mainly for chemical transformations[2] and bioassays[3].

Recently described biological activities of phytoecdysones on the differentiation of human kerotinocytes led to a patented design of their use in cosmetics and dermatology. Further necessary experiments required scaling up the production of 20-hydroxyecdysone and/or *Leuzea* ecdysteroid mixtures with fixed qualitative and quantitative compositions on a range of kilogram levels. The large-scale chromatography displayed a rich source of minor *Leuzea* ecdysteroids in until now unattainable quantities, as well as several new minors undetected in the previous low-scale separations. There were isolated and identified: isovitexirone, taxisterone, poststerone, 3-epi-20-hydroxyecdysone, already found and described in other species, and new minors with structures related to previously known constituents as are lactones: 24-hydroxy-dihydrocarthamosterone (*I*) and leuzeasterone (*II*).



I *II*

From some of the compounds a biogenetic relation has been derived. Certain previously described *Leuzea* ecdysteroids were not found in our material, which indicates geographic, seasonal or cultivar variations. However, our crude fractions contain still more minor compounds possessing characteristic ecdysteroid properties. Their purification and structural elucidation is in progress.

Supported by the Grant Agency of the Czech Republic, grant No. 203/98/0451.

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P57

CHEMICAL TRANSFORMATION OF ECDYSTEROIDS FOR ECDYSONE RECEPTOR MAPPING

JURAJ HARMATHA*, KAREL GRÜNER, VOLODYMYR MAŇKO, and MILOŠ BUDĚŠÍNSKÝ

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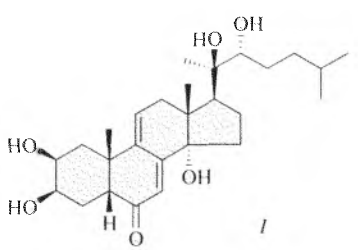
Biological activity of a series of more than 20 natural and semisynthetic ecdysteroids were compared in the B_{II} bioassay[1], which reflects the affinity of binding to the ligand binding-site of the *Drosophila melanogaster* ecdysteroid receptor. Natural compounds were isolated from plants (*Leuzea carthamoides*)[2] or fungi (*Paxillus atrotomentosus* and *Tapinella panuoides*)[3]. Structural analogues and conjugates were prepared by chemical transformation of 20-hydroxyecdysone[4]. The implication of the obtained data on the structure-activity relationship has been utilised to design further targeted chemical modifications, based on regioselective deoxygenations, dehydrations and phototransformations.



I

The *in vitro* method applied in the B_{II} bioassay allows investigate the binding site of the ligand-receptor complex under a variable UV light condition. Therefore structural modifications with a prolonged double bond conjugation, but with a minimum change in the characteristic conformation features were performed. Such compounds were expected to serve as photo-affinity labels with retained biological activity. Dienones obtained by dehydration of 11-OH or 14-OH from selected suitable ecdysteroids possessing such substituents served as models. Ajugasterone C was transformed to 7,9(11)-dien-6-one: dacryhainansterone (5-deoxykaladasterone) (*I*), described some time ago as a minor *Leuzea* or *Dacrydium* constituent (or artefact?). However, it was till now unavailable for a bioassay. Another way was to generate fluorescent derivatives (conjugates), but again with a retained activity. In our laboratory the 2-dansyloxy-20-hydroxyecdysone has been prepared and tested along with dansyl hydrazones, prepared for the same reason in the co-operating laboratory of the University of Exeter (U.K.) possessing the B_{II} bioassay.

Supported by the Grant Agency of the Czech Republic, grant No. 203/98/0451.

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P58

A NEW 3α-FLURO BRASSINOSTEROID DERIVATIVE USEFULL TO PROVIDE MORE INFORMATION ABOUT THE TYPE OF BRASSINOSTEROID-RECEPTOR INTERACTION

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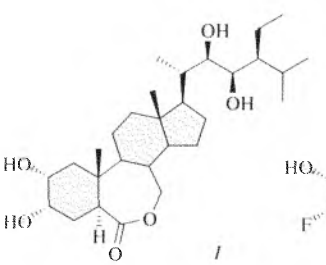
Brassinosteroids are potent plant growth regulators, which have an exciting potential use in agriculture for improving yield and quality of crops[1].

In our aim to define which are the most important functional groups of a brassinosteroid for expressing activity we



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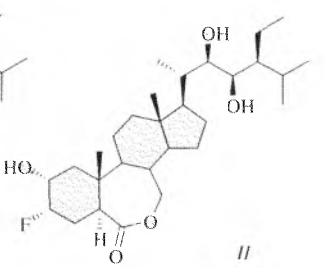

II

have developed a model based on molecular modeling techniques. The results obtained up to now suggests that the 3α-OH group of a brassinosteroid is more important than the 2α-OH group to elicit activity. In addition, due to the good correlation observed between the feasibility of hydrogen-bonding of this functional group and the activity we can assume that this type of interaction can takes place in the brassinosteroid-receptor complex[2].

Considering that the hydroxyl groups may act as an acceptor or donor of H-bounding, another interesting point to be determined concerns the clarification of how the 3α-OH group of brassinosteroids works on binding with the receptor.

Thus, the substitution of the hydroxyl groups of brassinosteroids for other functional groups working only as an accep-

tor or only as a donor of H-bond would be useful in providing a more precise information about how brassinosteroids interact with the receptor. In this communication, the substitution of the 3α-OH of 28-homobrassinolide (*I*) for a 3α-fluor giving *II* will be specifically analyzed from different points of view: molecular modeling, synthesis and bioactivity.

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P59
SYNTHESIS AND MOLECULAR MODELING OF A BRASSINOSTEROID ANALOG HAVING AN ANDROSTANE ESTER SIDE CHAIN

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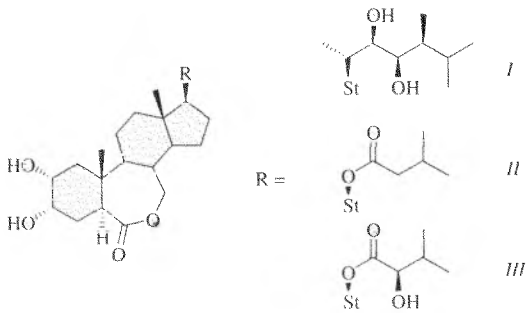
With the aim of looking for a more rigorous way to establish the structural requirements for a high brassinosteroid activity, a model based on brassinosteroid-receptor interaction has been described which is useful in explaining the activity of different brassinosteroids from the structural point of view[1]. Following this model, we have found that the electrostatic charges play an important role in explaining the activity and that the hydrogen-bonding could be one of the type of interaction that could take place on binding.



Based on the Grid methodology[2,3] over the set of brassinosteroids studied, the results obtained at present have allowed to provide defined information about the areas of the molecule responsible for binding and the ones eliciting activity[1]. The electronegative part of the side chain seems to be more important for exhibiting high activity that the one of the A-ring diol. Moreover, between the two hydroxyl groups of the side chain usually present in a brassinosteroid, the region with high probability of hydrogen-bonding near to the one of the 23*R*-OH of brassinolide (*I*) seems to be more important than the one of the 22*R*-OH group and essential for eliciting activity. This is in full agreement with the results obtained when the androstane brassinosteroid analog *II* is studied following the same procedure[4]. This compound has elicited only marginal activity in our rice lamina inclination test, at least at a dose lower than 2μg per plant, although *II* has been proved to be active in the bean second internode bioassay[5].

The lack of activity of *II* in rice lamina inclination test can be explained if one consider that its ester function presents only an area with high probability of hydrogen-bonding located near to the one of the 22*R*-OH of brassinolide (*I*), but slightly shifted to the right. No interaction is observed in the zone close to the 23*R*-OH of brassinolide (*I*).

With the aim to assess this and looking for new active brassinosteroid analogs with a good synthetic cost/activity relationship we are working on the design of new androstane brassinosteroid analogs having an additional functional group which fit to the area with high probability of hydrogen-bonding of the 23*R*-OH group of brassinolide (*I*).

On this communication, a new analog *III*, differing only to *II* for the presence of an extra OH in the side chain will be specifically analyzed. The synthetic strategy developed to obtain it, its feasibility of hydrogen-bonding by means of its Grid map, as well as its activity data will be presented and compared with those of *II*.

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P60
STUDY OF STRESS REACTIONS OF *IN VIVO* AND *IN VITRO NICOTIANA TABACUM* PLANTS INDUCED WITH METHYL JASMONATE

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Higher plants are responding to mechanical, pathogenic, herbivoral, and chemical stress by a sequence of biochemical events resulting in hypersensitive reactions, emitting of chemical messages to parasites of herbivores and to other plants, and in inducing of higher resistance towards future attacks[1]. However, not all the parts of this very complex event are understood in detail by now. In order to study, in part, the molecular basis of these stress responses we are developing a test system based on *in vitro* plants and corresponding plant cell cultures of *Nicotiana tabacum*. In this investigation we will mainly focus on the octadecanoid cascade.

The reaction of plants to the stress stimulation was observed on the *Nicotiana tabacum* plant (var. Wisconsin 38) belonging to the family Solanaceae. The detached leaves of plants and whole plantlets (with the first foliage leaves) without roots cultivated under sterile (*in vitro*) and non-sterile (*in vivo*) conditions, root callus and cell suspension were used. Methyljasmonate (MJ), in water solutions and/or in the form of vapors at concentrations 0.1–300 μmol l^{-1} was used as the elicitor of the stress[2].

We followed three classes of compounds produced by higher plants in response to stress. The production of volatile substances was assayed by a combination of SPME extraction and gas chromatography (GC), the peroxidase (POX) levels and isoenzyme patterns, and the changes of the protease inhibitors (PI) levels were measured by biochemical methods.

MJ-induced plants displayed dramatic changes in the production of the three terpenoids: (*E*)-ocimene, β-elemene and drima-7,9(10)-diene. The strongest responses were observed when the *in vitro* cultivated plantlets were induced with air-borne MJ at concentration as low as 100 nmol l^{-1}. In contrast to whole plants or detached leaves, the (*E*)-ocimene was not produced by the tobacco cell suspensions.

In the MJ-induced (100 μmol l^{-1}) plants cultivated *in vitro* total activity of POX was lower than in the control plants after 3 h of induction. When MJ was applied for a longer period (24–48 h) the POX activity had gradually increased. By native PAGE no induction of new POX-isoenzymes was detected. PI are inducible both in differentiated plants as well in cell cultures. It can be concluded, that *in vitro* plantlets and also less differentiated cultures are suitable for octadecanoid cascade studies.

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SYNTHESIS OF CALIX[4]PYRROLES AND PORPHYRINES WITH STEROIDAL MOIETIES

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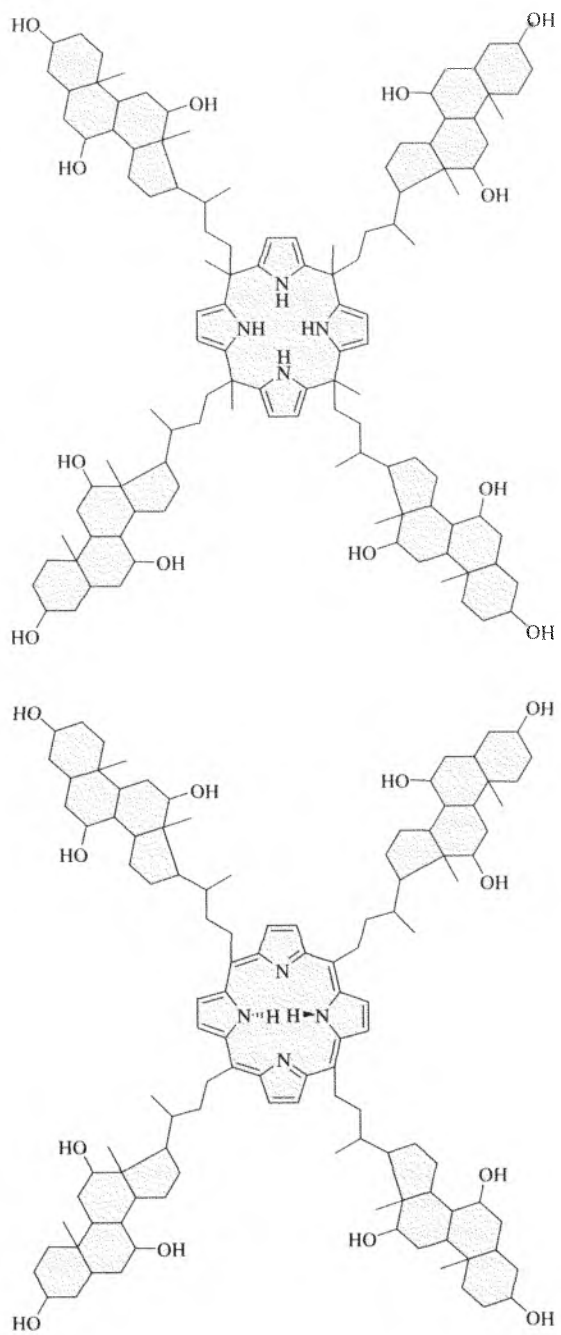
Calix[4]pyrroles (*meso*-octaalkylporphyrinogens) and porphyrins are gaining importance for their ability to take custom oriented role in suprasystems for ion complexation[1].

Present paper shows the possibility of utilization the condensation[2] of steroidal ketones of several different types with



pyrrole to calix[4]pyrroles and utilization of corresponding steroid aldehydes in the synthesis of porphyrins.

Thus, there were prepared several calix[4]pyrroles from steroid side chain ketones yielding into tetrasteroidotetramethyl-porphyrinogens of yet unknown stereochemistry. Reaction involves condensation of steroidal ketone and pyrrole in acidified ethanol. On the steroid part there were utilized derivatives of cholic acids and 20-homopregnenolone. Similar aldehydes were treated with pyrrole in dichloromethane with boron trifluoride etherate followed by oxidation by *p*-chloroanil yielding to tetrasubstituted porphyrins.

The study of physico-chemical and transport properties, complexation and stereochemistry is under progress.

The support of the Grant Agency of the Czech Republic, grants # 203/97/0695 and 203/97/1099 is greatly acknowledged.

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P62
ENANTIOSELECTIVE ALKYLATION OF CYCLANONES *VIA* CHIRAL LITHIOENAMINES

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An increasing demand for receiving enantiomerically pure compounds in any area of practical applications has encouraged a development of novel stereoselective methods of synthesis of chiral compounds, precursors and synthons. A stereoselective alkylation of cyclic and aliphatic ketones at the C(α) and C(α′) carbon centers has represented an efficient target of investigation of convenient methods of synthesis of both natural and biologically active compounds. Having dealt with a stereoselective synthesis of a series of insect juvenile hormone bioanalogs derived from α-substituted or α,α′-disubstituted cyclanones, we have applied a modified Michael-type addition reaction to introduce small alkyl groups stereoselectively into the molecules of racemic 2-(4-alkoxybenzyl)-1-cyclanones. Metalloenamines of chiral alkoxy amines have been found to introduce desired chirality into the system which has resulted in an asymmetric alkylation of the ketones at the C(α) or C(α′) carbon centers[1]. Amino acids have represented a convenient chiral synthons for preparation of chiral alkoxyamines with defined absolute configuration. We have employed (2*S*)- and (2*R*)-2-amino-3-phenylpropanoic acids to synthesize (2*S*)- and (2*R*)-2-amino-1-methoxy-3-phenylpropanes. Both methoxyamines bear key oxygen-containing functionalities, responsible for a metallo-ligand alignment with a fixed absolute configuration of the molecule. An attack of the electrophile to the C(α) or C(α′) carbon centers of the enamine has resulted in producing the alkylated products with controlled absolute configuration.

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P63
LIPASES – A TOOL FOR CHANGE OF COMPOSITION OF NATURAL TRIGLYCERIDES FROM THE BLACKCURRANT SEEDS

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A mixture of natural triglycerides has been enriched in α-linolenic acid and its glycerol derivatives either by a specific lipase hydrolysis or by a procedure consisting of a chemical hydrolysis and a subsequent selective enzymic esterification. The former reaction type has been provided in water at 40 °C with stirring (500 min^{-1}) during a 24 h period[1]. This procedure has been employed with the lipases from *Candida cylindracea*, *Candida rugosa*, *Mucor miehei* and *Pseudomonas fluorescens*, and with the immobilized lipases from *Mucor miehei*, *Pseudomonas cepacia* and *Candida cylindracea*. The reactions were provided in an isooctane/-phosphate buffer (0.1 M, pH = 7.0) mixture (2/1) at 30 °C under stirring at 500 min^{-1} for 4 h (ref.[2]). The immobilized lipase Lipozyme and those from *Pseudomonas cepacia*, *Mucor miehei* and *Candida cylindracea* were used. Employing the latter procedure of enrichment of a mixture of natural triglycerides towards α-linolenic acid and its glycerol derivatives[2], the chemical hydrolysis of triglycerides has been provided in a solution of potassium hydroxide in absolute ethanol at 80 °C for 90 min. A subsequent selective enzymic esterification with 1-butanol mediated by Lipozyme was provided in isooctane (under a presence of molecular sieves) at 30 °C for 2 h. A resulting mixture of fatty acids has been enriched in the content of α-linolenic acid. This fatty acid mixture has been

employed in Lipozyme mediated esterification of glycerol, provided in isooctane (under a presence of molecular sieves) at 50 °C and under stirring for 4 h.

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ENZYMIC FORMATION OF SELECTED ALKYL-*O*-GLYCOSIDES UNDER MICROWAVE IRRADIATION

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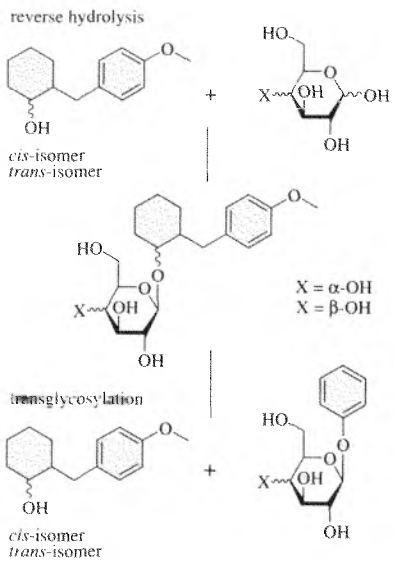
2-(4-Methoxybenzyl)-1-cyclohexyl-β-D-glucopyranosides and 2-(4-methoxybenzyl)-1-cyclohexyl-β-D-galactopyranosides, models for glycosidic juvenogens, were synthesized using either D-glucose or D-galactose [in their natural form or activated form (phenyl-β-D-glucopyranoside and phenyl-β-D-galactopyranoside)], and the respective racemic *cis* or *trans* isomers of 2-(4-methoxybenzyl)-1-cyclohexanol by either enzymic reverse hydrolysis or transglycosylation[1,2] under both, standard heating and microwave irradiation[3,4]. Commercially available β-glucosidase (EC 3.2.1.21) from almond or β-galactosidase (EC 3.2.1.23) from *Escherichia coli* were employed using different acetonitrile/water mixtures [9/1 (v/v) for the reverse hydrolysis, and 4/1 (v/v) for the transglycosylation]. The positive effect of the microwave irradiation on the chemical yield of the reaction was observed.



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P65
SYNTHESIS OF 7-METHYL-1,6-DIOXASPIRO-[4.5]DECANE

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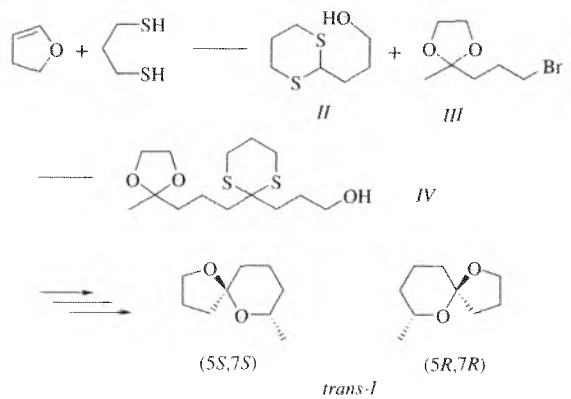
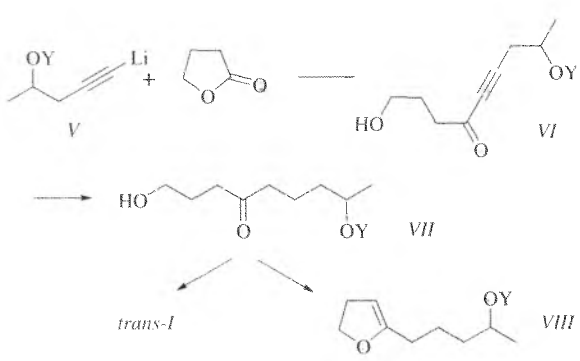
The synthesis of 7-methyl-1,6-dioxaspiro[4.5]decane (*I*) has been studied because of the potential role of this compound in chemical communication of the bark beetle *Scolytus*



Scheme 1

intricatus. The spiroacetal *I* is also known as a component of the volatiles produced by several wasp, bee and beetle species[1,2]. In spite of the relative simplicity of spiroacetal *I*, only a few synthetic[1,3] and structural[4] studies have appeared in the literature. The spiroacetal *I* can exist as four stereoisomers but only the two *trans-I* are well described.

The racemic *trans-I* was prepared on multigram scale by modification of the method reported previously[3] in 31% overall yield. Alkylation of the dithiane *II* by bromide *III* was followed by selective hydrolysis of the *O*-acetal *IV*, reduction of corresponding ketone and the thioacetal thus obtained was converted into the *trans-I* by treatment with CH_3I in aqueous acetonitrile (Scheme 1).



Scheme 2

In an effort to study the spirocyclization under kinetic and thermodynamic conditions[5] another approach was chosen (Scheme 2). The lithium acetylide of protected 4-pentyn-2-ol (*V*) (Y = THP, *t*BuPh$_2$Si) was reacted with γ-butyrolactone to give ynone *VI*. The hydroxyketone *VII* obtained after hydrogenation was converted either directly into the spiroacetal *trans-I* by deprotection (Y= THP, *t*BuPh$_2$Si) or into cyclic enol ether *VIII* by elimination of water (Y = *t*BuPh$_2$Si). The last mentioned compound provided after deprotection the desired hydroxyenol ether (Y = H) required for NMR study of cyclization. Also protected (*S*)-4-pentyn-2-ol was synthesised starting from methyl-(*S*)-lactate in five steps *via* a treatment (*S*)-1-iodo-2-propanol with lithium acetylide ethylenediamine complex.

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P66

SEMIOCHEMISTRY OF THE EUROPEAN OAK BARK BEETLE, *SCOLYTUS INTRICATUS* (COLEOPTERA, SCOLYTIDAE): A. CHEMICALLY MEDIATED BEHAVIOUR IN *SCOLYTUS INTRICATUS*; B. HOST PLANT VOLATILES FROM OAK (*QUERCUS ROBUR*) AND THEIR POSSIBLE ROLE IN *SCOLYTUS INTRICATUS* ATTRACTION

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A. The response of the European oak bark beetle (*Scolytus intricatus*) to chemical signals during the host selection and mating was studied using a walking arrestant-excitant bioassay and electroantennography (EAG). Significant differences in attractiveness were demonstrated for volatiles associated with the beetle-infested and non-infested oak sprouts and/or logs. Several additional trends were apparent: (1) The non-infested host material, while attracting a significant number of beetles, did not discriminate between sexes; (2) males exhibited a stronger response to female-infested oak logs (females making galleries) and sprouts (females in maturation feeding) than to non-infested ones, while the corresponding male-infested material attracted both sexes in a similar degree as did the non-infested one; (3) females responded to female-infested host plants too, but the response was less pronounced; and (4) the EAG response to volatiles associated with the female-infested, male-infested and non-infested hosts was markedly higher in the first case (with approximately the same sensitivity of the male and female antennae). Our findings support a hypothesis that the aggregation behaviour in *S. intricatus* is, at least in part, governed by a female-produced pheromone.

B. Volatiles emitted in different phases of the oak (*Quercus robur*) development (bark, unopened buds, young developing leaves, and blossoms) were analysed with the aim to find host plant attractants for *Scolytus intricatus*. Complex mixtures of aliphatic, aromatic, and terpenic compounds were identified in the samples. *E,E*-α-Farnesene was the main component of the bark sample. Alcohols of different types and esters of aromatic or fatty acids formed the main part of the volatiles of buds. After the buds opened to leaves, alcohols were no longer produced and corresponding aldehydes dominated in the sample.

Volatiles released from oak twigs and branches both during maturation feeding and constructing maternal galleries by *S. intricatus* were analysed, too. Most compounds found in the samples from females' and males' maturation feeding were identical. High contents of anisole, (*E*)-β-ocimene, α-copaene, and one unidentified sesquiterpenic hydrocarbon

($C_{15}H_{24}$) were found in both samples of twigs with feeding beetles. During the construction of maternal galleries by bark beetles in oak logs, monoterpene hydrocarbons such as α-thujene, *p*-cymene, limonene, and γ-terpinene, and sesquiterpene α-copaene were released in high quantities. No new compound appeared when males were added to the log with feeding females. Although a number of identified volatiles showed biological activity in preliminary EAG tests, they seem to belong to the host plant volatiles and not to signals produced by bark beetles.

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SYNTHESIS OF 17β-AMINOSTEROIDS

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Androstane derivatives substituted by cyclic amines in positions 2, 3, 16 or 17 were studied especially for their potential neuromuscular blocking activities[1].

17β-Aminoandrostanes are generally accessible by Leuckart–Wallach reductive amination of 17-oxoandrostane derivatives[2]. Since the enamine formation of 17-oxosteroids is rather difficult in the case of six-membered cyclic amines, high temperatures, prolonged reaction times and large excess of the amine are required in this procedure.

Hence, we aimed to investigate applicability of modern methods of reductive amination using borohydride reagents. Under Borch[3] conditions ($NaBH_3CN$/MeOH), piperidine, morpholine and *N*-methylpiperazine afforded virtually no 17-aminosteroids. To promote formation of the intermediate iminium adduct, a process which uses titanium tetrachloride[4] as Lewis acid catalyst and water scavenger was successfully employed. Finally, best results were achieved using titanium(IV) isopropoxide[5] mediated enamine/imine formation followed by sodium cyanoborohydride or triacetoxyborohydride reduction.

The study was supported by grant No. 203/97/0695 from the Grant Agency of the Czech Republic.

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P68
STEROID METABOLIZING ENZYME - 17β-HYDROXYSTEROID DEHYDROGENASE IN FILAMENTOUS FUNGI

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Mammalian 17β-hydroxysteroid dehydrogenases (17β-HSDs) are well known and characterized enzymes. They catalyze the interconversions of 17-hydroxy and 17-keto steroids in the presence of coenzymes NAD/H or NADP/H and are involved in the biosynthesis of estrogens and androgens as well as in the regulation of steroid hormone action.

17β-HSD activity was found also in microorganisms, bacteria, filamentous fungi and yeasts. While bacterial inducible HSDs have been already purified and characterized, much less is known about fungal 17β-HSDs. Although the constitutive 17β-HSD activity has been determined in filamentous fungi and yeasts, the representatives of different fungal classes, its physiological function is far from clear. To obtain more detailed information about these fungal enzymes constitutive 17β-HSDs from two fungal species: *Cochliobolus lunatus* and *Pleurotus ostreatus* were studied. Both enzymes have been purified and shown to have quite different characteristics. The enzyme from ascomycetous fungus *Cochliobolus lunatus* favours reduction of androgens and estrogens in the presence of coenzyme NADPH, whereas the enzyme from basidiomycetous fungus *Pleurotus ostreatus* preferentially catalyzes oxidations of androgens and estrogens in the presence of NAD^+. No significant 3α/β-HSD and 20α/β-HSD activity was detected in both enzymes using 5α-androstan-3-one and 4-pregnene-3,20-dione as substrates.

Furthermore 17β-HSD from *Cochliobolus lunatus* was investigated in more detail. The enzyme was cloned and expressed in *E. coli*. The cDNA and corresponding protein sequence that were determined show high similarity with several fungal ketoreductases involved in the biosynthesis of mycotoxins, aflatoxin and sterigmatocistin, and the fungal pigment melanin. Among HSDs, the highest similarity was found with 7α-HSD from *E. coli* involved in bile acid metabolism and even with human 17β-HSD type 4. Substrate specificity of recombinant enzyme toward potential steroid substrates was studied. Among tested steroids androgens and estrogens, reductions of 4-androstene-3,17-dione, 5α-androstane-3,17-dione, 4-estrene-3,17-dione and 1,3,5(10)-estratrien-3-ol,17-one were found to be most efficient, while reductions of 5α-androstan-3α-ol,17-one, 5α-androstan-3β-ol,17-one, 5α-androst-2-en-17-one, 1,4-androstadiene-3,17-dione and oxidations of 5α-estrane-3α,17β-diol, 5α-estran-17β-ol-3-on, 1,3,5(10)-estratriene-3,17β-diol, 5α-androst-2-en-17β-ol, 5α-androstane-3α,17β-diol and 1,4-androstadien-17β-ol-3-one, were less effective. There was however no conversion of 5β-steroids: 5β-androstane-3β,17β-diol, 5β-androstane-3,17-dione, 5β-androstan-3β-ol-17-one and 5β-androstan-17β-ol-3-one.

Further studies of steroid substrate specificity that might give us indications of physiological substrates and of endogenous function of fungal 17β-HSD, are currently in progress in our laboratory.

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STEROIDOGENESIS IN FILAMENTOUS FUNGI

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Hormones have generally been assumed to be exclusively the products of vertebrate endocrine organs. However, recent reports have shown that the generally accepted assumption of the unique connection of steroid hormones with mammals does not hold any more. Even unicellular organisms, *e.g.* protozoa and fungi, have been shown to contain components of the steroid hormone system such as primitive steroid receptors. In our laboratory the components of the steroid hormone signalling system: 17β-hydroxysteroid dehydrogenase (17β-HSD) and steroid binding proteins were identified in filamentous fungi *Cochliobolus lunatus* and *Pleurotus ostreatus*. Since exogenously added steroid hormones were found to be good substrates of 17β-HSDs in both fungi, the question arose whether the role of this enzyme in fungi could be similar to that in higher organisms. The present study is an attempt to elucidate the question of the endogenous substrate of the studied 17β-HSDs by actual isolation and identification of the putative steroid signalling molecule or molecules.

De novo synthesis of steroid hormones was demonstrated in *Cochliobolus lunatus* and *Pleurotus ostreatus* by the incorporation of radioactively labelled precursor molecule mevalonate into steroids. Steroids were further analysed by GC-MS. Results of our study show that synthesis of steroid hormones testosterone and androstenedione takes place in both fungi. The identification of an additional steroid testololactone in *Pleurotus ostreatus* suggests some differences in steroidogenesis in *Cochliobolus lunatus* in comparison to *Pleurotus ostreatus*. These results are consistent with our finding that 17β-HSDs in both organisms differ in some of the characteristics; while in *Cochliobolus lunatus* it preferentially converts androstenedione to testosterone in *Pleurotus ostreatus* it oxidizes testosterone to androstenedione which may be further converted to testololactone. Our study thus suggests diversity in endogenous steroidogenesis among fungi and implies possible differences in biological functions of steroidal molecules in fungi which still remain to be elucidated.

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FACILE ENTRY TO OXASTEROIDS *VIA* THE INTRAMOLECULAR CYCLOADDITION OF SUBSTITUTED o-QUINONEMETHIDES

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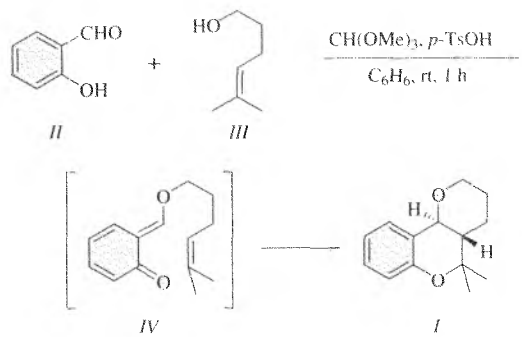
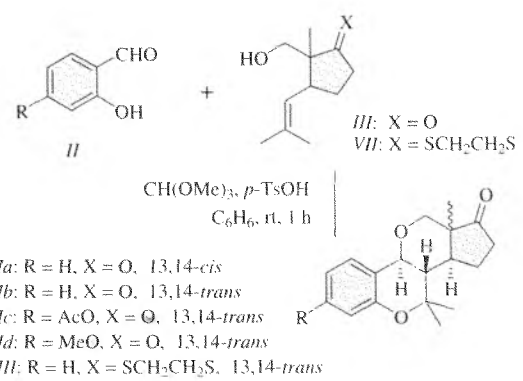
Heterosteroids are of paticular interest because of their enhanced or unique biological activities. Although many azasteroids have been reported hitherto, relatively few papers have appeared dealing with the oxasteroids[1]. Here we report a general method of construction of 6,11-dioxasteroid skeleton from salicylaldehydes and substituted cyclopentanone derivatives as the starting materials.



We reported a facile synthesis of 3,4,4a,10b-tetrahydro-2*H*,5*H*-pyrano[3,2-*c*][1]benzopyrans (*I*) by the one-pot condensation of salicylaldehydes (*II*) and unsaturated alcohol *III via* the intramolecular cycloaddition of 6-alkenyloxymethylene-2,4-cyclohexadien-1-ones (*IV*) at room temperature[2].



We anticipated that the dioxasteroidal skeleton will be formed using *II* and 2-(hydroxymethyl)-3-vinylcyclopentanone derivatives (*V*). Thus, the reaction of a 1 : 1 mixture of *cis*-2-(hydroxymethyl)-2-methyl-3-(2-methyl-1-propenyl)cyclopentanone (*Va*) and the *trans* isomer (*Vb*) with *II* in the presence of trimethyl orthoformate and a catalytic amount of

p-toluenesulfonic acid afforded two diastereomers *VIa* and *VIb* in 18% and 9%, respectively. When pure trans isomer *Vb* was used in the above reaction at reflux of benzene, pure *VIb* was obtained in 55% yield. Similarly were obtained 7,7-dimethyl-6,11-dioxaestrone acetate (*VIc*) and 7,7-dimethyl-6,11-dioxaestrone methyl ether (*VId*) from the correspopnding substituted salicylaldehydes and *Vb*.

In order to suppress degradation of thermally labile *Vb* during the reaction, *Vb* was converted to the dithioacetal *VII*, which was reacted with *II* in benzene at reflux to afford a mixture of dithioacetal *VIII* (25% yield) and *VIb* (36% yield).

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