ECDYSTEROIDS AND RELATED MOLECULES IN ANIMALS AND PLANTS

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Ecdysteroids are polyhydroxylated steroids firstly isolated from insects (1954) and later discovered in plants (1966). This family of molecules bears unique structural features (a cis-A/B ring junction, a 7-en-6-one and a 14α-OH) and it comprises more than 300 representatives1.

Zooecdysteroids are present in all Arthropods and represent “moulting hormones” which control in fact not only their growth (i.e. moult and metamorphosis) but also their reproduction. If 20-hydroxyecdysone is the most common ecdysteroid, some diversity is observed within arthropod ecdysteroids as concerns their number of carbon atoms (27 to 29) and the number and position of hydroxyl groups. The biosynthetic pathway has not yet been fully elucidated, and a “black box” remains concerning the early steps, but new approaches have recently allowed the full characterization of several biosynthetic enzymes belonging to the cytochrome P450 (CYP) family thanks to Drosophila developmental (halloween) mutants23.

Besides their classical endocrine roles, ecdysteroids may fulfill allelochemical functions in some Arthropods, and this can be exemplified by the marine Arachnid Pygomonan litorale which accumulates huge amounts of ecdysteroids in its integument as a defense mechanism against attack by crabs4, and the Chrysomelid beetle Chrysolina carnifex, which incorporates large amounts of ecdysteroids in its defensive secretions5.

Ecdysteroids have also been found in non-Arthropod Invertebrates including primitive Anthozoaans but, although their distribution and titer changes can in some instances be correlated with developmental events, and although exogenously applied molecules may have clearcut effects, attempts to demonstrate their endogenous origin have so far been unsuccessful.

Primitive Invertebrates (e.g. Sponges) contain a wide array of polyhydroxylated steroids, including true ecdysteroids, the origin of which need to be determined6. Whether some of these polyhydroxylated steroids are biogenetically related to ecdysteroids (= proto-ecdysteroids?) and how the Arthropod ecdysteroids have appeared is an important open question. Molecular approaches on biosynthetic enzymes and/or receptors will perhaps provide some answers in a near future.

Phytoecdysteroids have been found in ca. 6 % of the analyzed plant species (Ferns, Gymnosperms, Angiosperms) and in a few species of fungi; ecdysteroid-related molecules (pinnasteroids) are present in some Algae too7. Although structurally related to brassinosteroids, ecdysteroids have no established physiological role in plants, and they represent secondary metabolites able to protect plants against phytophagous Insects (and soil Nematodes?) through toxic and/or deterrent effects8,9.

The diversity of phytoecdysteroids is much higher than that of zooecdysteroids, but this could be a simple consequence of their high levels in plants (their concentrations may reach 30 g kg⁻¹ dry weight), which allows their more convenient isolation. A single plant species contains in fact a complex ecdysteroid cocktail10. Their biosynthetic pathway is not better known than that of Insects, but from an evolutionary point of view it would be of great interest to elucidate it and thus to determine whether both proceed through the same steps. Ecdysteroid distribution within plant organs does not follow a common pattern – they accumulate mainly in underground or aerial parts, in stem bark, flowers and/or seeds, … – and within a given specimen this pattern may change during ontogeny, which raises fundamental questions concerning the site(s) of production and the transport systems within the plant. These questions will be illustrated with the spinach Spinacia oleracea11.

Such a survey would not be complete without describing the pharmacological effects of ecdysteroids on Mammals and Humans. Soon after the discovery of phytoecdysteroids, and probably in connection with the idea of using them as pestici-des, ecdysteroid effects were tested on Mammals (in vivo and in vitro). These studies showed, besides a very low toxicity (LD50 > 6 g kg⁻¹ body weight in mice), a wide array of pharmacological actions12 among which we will essentially comment the anabolic and hypoglycemic ones. Interestingly, several plants used in traditional medicine belong to the ecdysteroid-rich species (e.g. Leuzea carhamoides, Pfaffia paniculata, Ajuga iva, …), and their effects could be explained (at least in part) by their high ecdysteroid content.

Finally, what are the practical uses of ecdysteroids? A first use connected with their physiological role in Insects concerns the improvement of silk production13 and of honeybee health14. A second use concerns the development of inducible gene expression systems, both in vitro with cell cultures (i.e. for fundamental research on gene function) and in vivo (with the aim of developing gene therapy systems)15. A third use is related with the above-described anabolic effects, and indeed there is a tremendously developing offer on the web of ecdysteroid-containing preparations for sportsmen and bodybuilders16. Natural ecdysteroids have no development in agriculture at the moment, however synthetic non-steroidal ecdysteroid agonists prove efficient in the field17; a better understanding of ecdysteroid biosynthesis and of its regulation will possibly allow new strategies for crop auto-protection to be developed in the future.

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METABOLIC CONVERSIONS OF DEHYDROEPiANDROSTERONE (DHEA) TO NEW ACTIVE STEROIDS: STRUCTURE / ACTIVITY RELATIONS

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The C_{19} steroids are derived from cholesterol via pregnenolone to a main source, 3β-hydroxyandrost-5-en-17β-one, commonly referred to as DHEA. This molecule was first identified by Butenandt and Dannenbaum who isolated it from male human urine as the 3-chloro substituted steroid and recognized that the halogen had been introduced by treating a urine fraction with HCl. The chemical properties of DHEA have been and continue to be, actively investigated by several eminent Czech scientists. The physiological actions of DHEA were pioneered by Dr. Jeri Sonka of Charles University who summarized ten years of his work in a useful monograph.

Because DHEA has many desirable physiological properties but displays these only weakly, we began to search for metabolites that might be more active than this parent steroid. There were many known metabolites to be examined and we developed an assay with which to measure their relative activities. Like the thyroid hormone, DHEA induces the formation of liver mitochondrial glycerophosphate dehydrogenase (GPDH) and cytosolic malic enzyme (ME) when fed or injected into rats and this assay provides a semi-quantitative measure of one activity of these steroids.

Hydroxylation of DHEA at any position other than 7 resulted in complete loss of activity, but 7α-hydroxy, 7-oxo-, and 7β-hydroxy DHEA were progressively more active than DHEA. Among inhibitors tested, glycyrrhetinate, an inhibitor of 11β-hydroxy steroid dehydrogenase, decreased the activity of the 7α-hydroxy and 7-oxo derivatives but enhanced that of 7β-hydroxy DHEA. This will be discussed below.

DHEA incubated with rat liver homogenate fortified with ATP, NADPH and malate is converted to some 40 different steroids. Repeated sampling and analysis of products formed at short time intervals disclosed the conversion of DHEA to 7α-hydroxyDHEA, to 7-oxoDHEA, to 7β-hydroxyDHEA in sequence. Half of the DHEA accumulates as androst-5-ene-3β,17β-diol (Adiol) thus confirming findings of Schneider and Mason with rabbit liver. DHEA is hydroxylated at position 7α by the known P450 7B. 7α-HydroxyDHEA is oxidized by 11β-hydroxy steroid dehydrogenase (flip orientation) to produce 7-oxoDHEA. 7-oxo-DHEA (in reverse orientation) is subject to reduction by the same enzyme to produce 7β-hydroxyDHEA. These steroids are subject to sulfation at position 3β and to reduction at position 17. Sulfation then also occurs at 17β. The ability to induce the formation of rat liver GPDH and cytosolic ME increases in the order of synthesis: DHEA < 7α-hydroxyDHEA, < 7-oxoDHEA, < 7β-hydroxyDHEA indicating they are on their way to become an active hormone. 7-OxoDHEA is far more effective than DHEA as an enhancer of memory in old mice. While these in vitro conversions are very rapid and involve relatively large quantities of steroids, we must recall that although DHEA is the most abundant steroid in human blood plasma, less than 1 % of the total circulates as the free steroid. And it is the free steroid that undergoes the metabolic transformations. The low concentration in vivo limits all of the conversions to other active molecules.

Like rat liver, isolated mouse adipocytes (3T3-L1) also convert DHEA to a variety of steroids; the yield of Adiol is 70 % of the added DHEA and it is excreted from the cells to the aqueous medium. The formation of Adiol is important because Adiol possesses androgen activity that is not inhibited by hydroxyflutamide or bicalutamide, two agents that are commonly used to treat prostate cancer.

Human prostate cancer usually responds to anti-androgen therapy by undergoing a period of remission of several months to a few years. It then renews growth that is not suppressed by the traditional drugs. This final period is termed androgen-independent or hormone refractory.

Thus it is possible that the reason growth of some prostate cancers becomes resistant to anti-androgen agents is that Adiol is the androgen that is stimulating their growth. Therefore it is important to find agents that inhibit the androgen activity of Adiol as well as of testosterone and dihydrotestosterone. Two steroids bearing ethynyl groups at position 17α have some ability to thwart the androgen activity of Adiol and recent work has led to even more effective compounds. For example, 3β-acetoxyandrost-1,5-diene-17-ethyleneketal (ADEK) is an effective inhibitor of the androgenic activity of Adiol as well as that of dihydrotestosterone. The agonist effect of ADEK is...
less than that of hydroxyflutamide and therefore is less likely to induce withdrawal response in prostate cancer patients.

The metabolic conversion of steroids to more active structures beyond 7β-hydroxyDHEA has not yet been defined. There are several sulfated esters and glucuronides produced but the ones we have tested are not highly active. Hydroxylation at position 16a is especially prominent in children. 16α-, -16β-, and 16α,16β-Dihydroxysteriods 5-ene-7,17-dione and 3β,7β,16α-trihydroxyandrost-5-ene-17-one are as active as 7-oxoDHEA. Likewise, 3β,16α,17β-androstene-triol is inactive but introduction of an oxo group at position 7 restores activity. Thus it appears that if the active hormone produced from DHEA carries oxygen at position 16, that oxygen must be introduced after 7 is oxygenated. Expanded A or B ring derivatives of DHEA are inactive but 3β-acetoxy-17α-oxa-androst-5-ene-7,17-dione is fully active. There are many products formed in liver tissue from DHEA that have not yet been completely characterized. They are the basis of our present work.

REFERENCES

SYNTHESIS OF 15β-SUBSTITUTED STEROIDS

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Many biologically active steroids possess hydroxy groups linked at the D-ring methylene units, e.g. at the C-15 and C-16 positions. In recent years a lot of methods were proposed for D-ring oxy functionalization. Our interest was to devise a methodology to synthesize steroids with hydroxy(alkoxy)alkyl moiety at C-15 position.

In this communication we discuss the application of androst-15-en 3-ethers such as 1 for the introduction of 15β-substituent containing oxygen. The title compounds were prepared according to the following scheme.

Details of preparation and identification procedures will be discussed.

It should be noted that using of the obtained compounds 9–12 in ene reaction opens a way to the corresponding 15β-substituted derivatives of pregnane and cholestane series.

REFERENCES
A STEREOSELECTIVE APPROACH TO THE BRASSINOLIDE SIDE CHAIN via 22-ISOXAZOLINYLSTEROIDS

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As a part of our study on the use of nitrile oxide methodology in the synthesis of biologically important steroids we now wish to report a new procedure for obtaining of the $22^R,23^R$-dihydroxy-24$S$-methyl functionality based on the stereoselective conversion of C$^{22}$-aldehyde I into the isoxazoline III possessing three asymmetric centers with the chirality desired for brassinolide.

Aldehyde I treated with organomagnesium bromide (obtained from cis-1-bromo-1-propene and magnesium in THF under inert atmosphere) furnished the $22^R$-hydroxy-(23Z)-olefin II in 70% yield. 1,3-Dipolar cycloaddition of acetonitrile oxide (generated in situ from corresponding acetaldimine, N-chlorosuccinimide and triethylamine in chloroform) to allyl alcohol II proceeded slowly to give a cycloadduct III (25% with 70% returning of starting material). Others diastereomers and regioisomers have not been detected in the crude mixture by NMR spectroscopy.

Taking into account that four diastereomeric products could be formed in this reaction the stereochemistry of the isoxazoline III have been determined by X-ray structure analysis. Some spectral and X-ray data, reaction mechanisms and chemical transformations of the isoxazolinylsteroid III into steroids with open side chain like IV will be discussed.

SYNTHESIS OF ISOXAZOLE ANALOGUES OF ECYDSTEROIDS

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Earlier we have shown that the application of isoxazolinylsteroids as key compounds allows effectively to form the
side chain of ecdysteroids, in particular ponasterone and pte-
rosterone C (ref.1). In this work we have put the purpose to
investigate an applicability of application 20-hydroxy-20 iso-
xazolinylsteroids such as I in reactions of formation of a cyclic
moiety of ecdysteroid molecule.

The proposed methodology is based on realization of the
fact that the heterocyclic ring is stable in many reactions that
allowed us to obtained a number of isoxasoline derivatives 2–7.
It was surprising the aromatization proceeding under action of
Lewis acids and resulting in isoxasole 8.

Evidence for the structures 2–9 was obtained by spectral
methods; details will be discussed.

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PALLADIUM- AND NICKEL- CATALYZED
CROSS-COUPLING ARYLATION IN A SERIES
OF 4- AND 6-HALOGEN SUBSTITUTED STEROIDS

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It is known that some of 4-and 6-substituted derivatives of
androstene and 17-hydroxyprogesterone can act as aromatase
or 5-α-reductase inhibitors, posses contraceptive or other use-
ful type of activity. During our research in modification of
complex organic molecules by Pd-catalyzed cross-coupling
reactions we found an easy and convenient approach to 4- and
6-aryl substituted steroids by cross coupling of 4-bromoan-
drost-4-ene-3,17-dione, 4-bromo-17-hydroxyprogesterone
and 6-chloromadinone acetate with arylboronic acids. Usually
aryl- and vinyl bromides are convenient substrates for substi-
tution of bromine to aryl group in Suzuki reaction. In some
cases spatially hindered substrates like steroids are reluctant
to take part in such reactions. However we have not meet any
problems with arylation of above mentioned 4-bromosubsti-
tuted steroids under standard conditions giving the respective
products in high yields.

Cross-coupling with spatially hindered vinyl chlorides is
a more difficult task. We have studied an influence of cata-
lysts, solvents and bases on the yields of 6-amisylmadinone
acetate and found the best conditions, providing a series of
some of 6-aryl substituted derivatives in yields from moderate
to quantitative.

4-Carboxyphenyl substituent was found to be a convenient
spacer group for binding above mentioned steroid molecules
to protein carriers to prepare immunogens.
Determination of Linear Terpenes Enantiomers Present at Low Quantitites in Natural Ultri-Compound Mixtures

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Chiral linear terpenic alcohols such as citronellol, 2,3-dihydrofarnesol and geranylcitronellol play an important role in interspecies chemical communication of bumblebees. These compounds were also identified in scents of several flowers that are pollinated by bumblebee as well. It is important to know the ratio of enantiomers in chiral natural products. In many cases, interactions between receptor proteins in insect antenna with just one specific enantiomer of pheromone constituent were proved.

The composition of secretions produced by bumblebee’s labial gland (LG) and the scent of flowers was identified by gas chromatography with mass spectrometry detector. The structure confirmation was done by comparison of mass spectra the NIST library and the standards.

For separation of the linear terpene enantiomers from extracts of male LG and from scent of orchids, a two-dimensional gas chromatographic (2D-GC) technique was used. This technique is crucial e.g. in case of determination the enantiomeric ratio of key monoterpenes in oviposition attractants of the Cameraria ohridella host plant Aesculus hippocastanum. This system represents an enantiospecific reaction between antennal receptor proteins of pest moth C. ohridella and kairomone of the horse chestnut.

Stationary phase in chiral column for separations of linear terpenic alcohols was 60% of (3R,2S)-citronellol and (3S,2S)-2,3-dihydrofarnesol was reached at the temperature around 110 °C. Higher pressure of carrier gas was used to reduce extensive retention time of 2,3-dihydrofarnesol.

At these conditions, the retention time of citronellol was 21 minutes and 2,3-dihydrofarnesol was 131 minutes.

Pure (−)-(S)-citronellol and (−)-(S)-2,3-dihydrofarnesol, respectively, were found in the LG of all investigated bumblebees and cuckoo-bumblebees: Bombus terrestris, B. lucorum, B. jenellus, Psithyrus impatiens, P. bohemicus, and P. pyrenaes as well as in the volatiles collected from Orchis pauciflora, O. boryi, and Barlia robertsiana. This finding may indicate a narrow relationship between non-rewarding orchids and their pollinator bumblebees. A hypothesis of flower mimics of the bumblebee male pheromone for attracting pollinators will be discussed.

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STUDIES ON BRASSINOSTEROIDE HORMONE BINDING PROTEINS FROM PLANTS

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Brassinosteroids (BRs) are a group of plant steroids, of which the molecular and biochemical analysis of Arabidopsis mutants has furnished conclusive evidence that these compounds are plant growth hormones. They are biologically active in the various bioassay systems designed for gibberellins, auxins and cytokinins, eliciting remarkable growth responses. The molecular mechanism of BRs 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. Recently published opinion is, that BRs act in plants after binding to a sterol binding protein (SBP), which complexes with receptor in the membrane, but also binding of BRs to the membrane receptor alone is not excluded.

In order to isolate the BRs and oxysterol-binding proteins or receptors we prepared brassinosteroid-based bioaffinity ligands. Affinity chromatography carrier matrices obtained by oriented immobilisation of BRs ligands bound covalently by a proper spacer arm were compared for performance with plant extracts. The columns were used to isolate enough proteins from plant extracts for sequencing. Just now the final goal is cloning of receptors using methods of reverse genetics. The ligand must be bound by that part of the molecule which least participates in the biospecific binding. As far as it is not yet known exactly, which parts of the BRs molecule are actually necessary for the proper biological activity and which ones for specific binding, oriented immobilisation of different ligands to matrix was necessary. Among others newly synthesi-
sed brassinosteroid, (20S)-2α,3α-dihydroxy-7-oxa-B-homo-5α-pregn-6-one-20-carboxylic acid, was used for immobilisation. This compound was obtained in eight steps by general synthesis of brassinosteroid skeleton from bisnorcholanic acid. Various new BRs derivatives, tested also for other activities, were used to obtain chromatography carriers with BRs bound through the ring A, ring B or the side chain. Different carriers were tested to obtain acceptable yield of proteins in amounts sufficient for analysis of their primary structure.

The plant extracts were obtained by grinding frozen plant leaves or callus tissue, salts were removed by gel filtration and the extract applied to the bioaffinity matrix. The analysis of primary structures by sequencing of proteins obtained is underway. One of the proteins separated from Nicotiana tabacum callus extract was identified as osmotin-like protein pre-cursor. The aminoacid sequence obtained showed a 100% agreement with this pathogen-related (PR) protein, which appears in tobacco under stress conditions. Our results thus reveal a connection between brassinosteroid and protein involved in stress response.

The work was sponsored by grant A4055204 of the Grant Agency of the Academy of Sciences of the Czech Republic and research project Z4 055 905.

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The steroids synthesized by the brain and nervous system, named neurosteroids, have a wide variety of diverse functions. In general, neurosteroids mediate their actions, not through classic steroid hormone nuclear receptors, but through other mechanisms such as through ion gated neurotransmitter receptors. In the adult, neurosteroid stimulation of neurotransmitter receptors results in behavioral effects, such as decreased an-
xiety, sedation, and decreases in seizure activity, these effects being associated with stimulation of neuronal GABA_A receptors (for review, see^{1-3}). Thus, one of the primary neurosteroid target receptors considered is the γ-aminobutyric acid type A (GABA_A) receptor complex.

The GABA_A receptor/Cl^- ionophore complex is an oligomeric protein that has separate but allosterically interacting binding sites for the endogenous neurotransmitter GABA, for benzodiazepines and for picrotoxin-in-like convulsants. Known positive allosteric modulations include the enhanced binding of benzodiazepine agonists by GABA, the enhanced GABA-induced Cl^- flux by benzodiazepines and barbiturates and the different modifications of [35S]t-Butylbicyclicphosphorothionate binding induced by GABA, benzodiazepines and barbiturates. Certain pregnane steroids produce clear behavioural effects including, anxiolysis, sedation, anaesthesia and are anti-convulsant. This behavioural profile is characteristic of compounds that act to enhance the actions of GABA acting at the GABA_A receptor. It was first shown that the neuroactive steroids 3α,5α-tetrahydrodeoxy corticosterone and 3α,5α-tetrahydroprogesterone (3α-hydroxy-5α-pregn-20-one or one allopregnanolone) enhanced the binding of muscimol and benzodiazepines to GABA_A receptors, enhanced the GABA-elicited Cl^- current and displaced TBPS binding. All these effects were consistent with neuroactive steroids acting as positive modulators of GABA_A receptors and, hence, modulating neuronal excitability in the nervous system (for review, see^{4}).

Numerous synthetic steroids have been synthesized in an attempt to therapeutically exploit the behavioural effects of the pregnane steroids. The conversion of steroid derivatives with better pharmacological profiles has to be considered when evaluating the putative clinical properties of neuroactive steroids in vitro. Primary cultures of cortical neurons, constitutively expressing GABA_A receptors, are a good in vitro system model to study allosteric interactions at the GABA_A receptor\(^{4,7}\). The increase of Cl^- flux or the increase of [3H]flunitrazepam binding produced by a compound in this in vitro system may be predictive of the in vivo action of this compound as positive GABA_A receptor allosteric modulator. In this work we have determined the effect of several newly synthesized pregnane derivatives on [3H]flunitrazepam binding. In an attempt to increase the stability of the pregnane derivatives, a fluorine atom was introduced in position 3. Primary cultures of cortical neurons were used to assess the effects of these newly synthesized pregnane derivatives on inhibitory GABAergic neurotransmission.

Preparation of primary cultures of cortical neurons from 16-day-old mice fetuses and performance of [3H]flunitrazepam binding was performed as described elsewhere\(^{6,8}\).

Allopregnanolone (compound A) increased [3H]flunitrazepam binding in a concentration-dependent manner with an EC50 value of 1.35 μM. Substituting 3α-OH by a F atom led to a compound that did not increase [3H]flunitrazepam binding. Further structural modification of this 3-F derivative produced compounds with different effects on [3H]flunitrazepam binding. Introduction of an OH group in position 2 (compound B) produced a compound that slightly increased [3H]flunitrazepam binding, partially recovering the effect of allopregnanolone. However, introducing a longer acidic aliphatic chain produced two compounds (C and D) that even decreased [3H]flunitrazepam binding by 20–25%. The accompanying Fig. 1 shows the effects of these compounds.

According to the proposed hypothesis, none of the fluorine synthetized compounds would have a positive allosteric action in the GABA_A receptor similar to that produced by epalon. From the compounds tested, compound B may have a positive action on GABA_A receptor, however its efficacy is lower than that of epalon, and C and D compounds may directly interact with the benzodiazepine binding site. Other factors, like bioavailability and pharmacokinetics among others, should also be considered to establish the pharmacological interest of this compound.

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REFERENCES

ANALYSIS OF BILE ACID DERIVATIVES BY INHIBITION OF Na⁺/K⁺-ATPase AS TO THEIR POSSIBLE CARDIOACTIVITY

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The Na⁺/K⁺-ATPase – the molecular point of attack of cardioactive steroids – has been shown by us to be suited for the analysis of steroids of different types as to their respective proper-ties. Cholanic acids (bile acids) have the favourable 5β configuration and a 17β substituent with the same number of carbon atoms as the highly cardioactive bufadienolides. Therefore, they are potential and available starting compounds for the synthesis of cardioactive drugs despite of the less favourable 3α-OH and 14α-H (= C/D-trans connection). We have investigated 9 cholanic acid methyl esters (CA) carrying up to 3 OH groups and one amide with respect to their inhibition of human kidney Na⁺/K⁺-ATPase (Table I).

The Na⁺/K⁺-ATPase (human kidney) inhibition test

Equilibrium and kinetic constants1–4–6 of substances in the Na⁺/K⁺-ATPase (human kidney) inhibition test. k_on or k_off = velocity constant for formation or decay of the effector-receptor complex, respectively. K_D = k_off/k_on = inhibition constant at 37 °C and pH 7.4. sl = solubility, r.a.= relative activity (1 = 100).

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</tbody>
</table>

⁎No inhibition up to solubility limit

Compound 1 has only one equatorial 3-OH group which is common to all investigated CA except 2. Introduction of a second OH group increases [4: 6α- (eq), 6: 7α- (ax)] or decreases [7: 7β-OH (eq)] the activity compared to 1, whereas 6β-OH (ax) in 5 has no influence. Thus, improvement of the activity is caused by one OH-group at the α-face of the steroid backbone. A third additional OH group [9: 6α- (eq), 7α-OH (ax)] or [10: 7α- (ax), 12α-OH (ax)] did not further increase but decreases the activity. The 3α-O-acetyl-Δ¹ derivative of 1 (2) shows a strong decrease of activity. Exchange of -COOH in 1 against -CONHCH₂-COOH (3) shows nearly no influence on the activity. This is remarkable, as O→N exchange in the lactone rings of cardenolides2 or bufadionolides shows a strongly decreased activity of the lactames compared to the oxygen analogues. Compared to the cardioactive compounds bufalin1 or digitoxigenin1 or carnone1, the activity of 1 is about 4 or 3 orders of magnitude lower or one order higher, respectively. These differences reflect the differences in the velocity of the ATPase effector receptor complex formation (k_on) whereas the velocities of complex decay (k_off) are similar.

Most of other C/D-trans-steroids without 5β configuration show a more or less strongly decreased activity compared to 1.

The solubility of 1 in the measuring buffer solution is about 15 µM and increases roughly with an increasing number of OH-groups.

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A FACILE SYNTHETIC APPROACH TO CYCLOPENTACYCLOOCTANE DITERPENOID SKELETON USING RING-CLOSING METATHESIS

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The dicyclopenta[a,d]cyclooctene ring system is the structural core of numerous diterpenes (such as the fusicoxacin) and sesquiterpenes (such as the ophiobolins and ceroplastin). The wide range of biological activities exhibited by these compounds and their specific structural features have stimulated interest in developing synthetic approaches to their core ring system. In this communication we present a new approach to synthesis of 5–8 ring carbon framework using three consecutive ringclosure due to stereochemical factors will be discussed.
Human and murine species are able to hydroxylate DHEA at 7α-position and the presence of product of this hydroxylation (7α-hydroxy-DHEA) was observed in liver, brain and other tissues. The 7α-hydroxylated derivative has stronger biological activity than DHEA and shows more effective activation of immune processes in mouse. It enhances the resistance against lethal infections and counteracts the glucocorticosteroid immune suppression in peripheral tissues.

We have studied microbial transformation of DHEA. Three types of reactions were observed: hydroxylation, Baeyer-Villiger oxidation and reduction of carbonyl group.

7α-Hydroxy-DHEA was produced from DHEA by the fungi: *Absidia coerulea*, *Mucor hiemalis*, *Mucor circinelloides*, *Penicillium frequentans*, *Fusarium culmorum*, *Fusarium oxysporum*, *Nigrospora oryzae* and *Aspergillus ochraceus* while androstenedione was transformed to 6β-, 14α-, 15α-monohydroxy derivatives.

In some of the fungi used in present study, DHEA underwent the Baeyer-Villiger oxidation to the testolactone (*Penicillium camemberti*, *Penicillium lilacinum*) and to lactone with 3β-hydroxy-5-ene functions (*Penicillium camemberti*). 5-Androstene-3β,17β-diol, a known metabolite of nervous system tissues, was formed from DHEA in the *Botrytis cinerea* culture.

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NEW BRASSINOSTEROID ANALOGS HAVING NITROGENATED FUNCTIONALITIES AT C3 TO PROVIDE MORE INFORMATION ABOUT THE BRASSINOSTEROID-RECEPTOR INTERACTION

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

Considering that hydrogen bonding interaction can take place in the brassinosteroid-receptor complex, interesting points of view to be determined are: (1) whether the OH groups present in an active brassinosteroid act as acceptors or as donors in such hydrogen bonding, (2) the contribution of each OH group presents in the brassinosteroid to develop biological activity.
In this sense and focused on the A ring, the substitution of the OH function at C3 by another functional group, amine or azide and the activity evaluation of such compounds should give us more information about the type of interaction that could take place upon binding in such positions. Moreover, analogs with the presence or not of an adjacent OH at C2 would be useful to extend the number of compounds with modifications on the A ring and useful to clarify the contribution of OH-C2 and OH-C3 in the activity.3

In this communication, we present the synthetic strategy and bioactivity evaluation in the rice lamina inclination test (RLIT) towards different brassinosteroid derivatives with an amine or azide function at C3 on the A-ring, having or not OH function at C2.

This work was supported by a grant from Generalitat de Catalunya (No. 2003 F1 00930). M. M. is grateful to Generalitat de Catalunya for a fellowship.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF HETEROCYCLIC STEROIDS: TARGETING INHIBITION OF CYTOCHROME P450 ENZYMES IN BREAST AND PROSTATE CANCERS

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The cytochromes P450 constitute a super family of heme-thiolate enzymes, present in all species. The P450 enzymes involved in steroid hormone biosynthesis represent an important target for drug discovery and development for the treatment of hormone-dependent cancers (reviewed by Van Wauwe and Janssen). The most extensively developed are the inhibitors of the enzyme cytochrome P450 aromatase (CYP19), responsible for the conversion of androgens into estrogens and a target in the treatment of breast cancer. The sequence of reactions catalyzed by CYP19 involves three sequential enzymatic hydroxylations (Scheme 1). The first two take place on the C-19 methyl group, whereas the final hydroxylation step is still unclear.

Because ~80% of patients with prostate cancer have androgen-dependent diseases, that respond to hormonal ablation, the inhibition of androgen synthesis is also an important target for the treatment of prostate cancer. The last step in the biosynthesis of androgens involves the two-step conversion of pregnenolone and progesterone, via their corresponding 17α-hydroxy derivatives, to dehydroepiandrosterenedione and androstenedione respectively. Both reactions are catalyzed by the same enzyme; cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17) (Scheme 2).

Extensive worldwide efforts have resulted in the identification of structurally diverse series of inhibitors of CYP19 and
Indeed, four CYP19 inhibitors, namely, 4-hydroxyandrostenedione (formestane), anastrazole, letrozole and exemestane are used clinically for the treatment of breast cancer (reviewed by Njar and Brodie). A number of CYP17 inhibitors are currently in development as potential agents for the treatment of prostate cancer. The present contribution will focus on our efforts in the rationale design, synthesis and evaluation of inhibitors of CYP19 and of CYP17. Specifically, we describe studies of steroid inhibitors, involving functionalization (N-heterocycle) at either the C-19 (for CYP19 inhibitors) or the C-17 (for CYP17 inhibitors) positions in such a way as to mimic the natural substrates of the respective enzymes. The overall strategy is aimed at producing substrate-like compounds which are likely to not only interact with the steroid binding site of the enzyme, thus introducing high specificity, but also to provide a sixth ligand to the enzyme’s heme iron resulting in tight binding.

Aromatase (CYP19) Inhibitors: The novel (19R)- and (19S)-10β-aziridinylestr-4-ene-3,17-diones 1 and 2 and the corresponding (19R)- and (19S)-10β-aziridinyl-17β-hydroxyestr-4-ene-3,17-diones 3 and 4 (Scheme 3) have been prepared from the 19-oximino-19-methyl intermediate. The key reaction was the conversion of the 19-oxime into the diastereomeric 10β-aziridines by lithium aluminium hydride (LAH). The synthetic route used is briefly summarized in Scheme 3. Rigorous establishment of the C-19 configuration in the aziridinyl steroids was secured by the X-ray crystallographic analysis of compound 3. The compounds were shown to be powerful and stereoselective inhibitors of human placental microsomal aromatase. We also showed that the nitrogen atom of the most potent aziridine coordinates to the enzyme’s heme iron. These compounds are amongst the most potent inhibitors of this enzyme to date.

CYP17 Inhibitors: In our search for potent and selective inhibitors of CYP17, a variety of novel Δ16-17-azolyl steroids, that is, pyrazoles, imidazoles, triazoles and tetrazoles have been prepared by a new synthetic route. This involved the nucleophilic vinylic “addition-elimination” substitution reaction of 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene and azolyl nucleophiles. These series of azolyl steroids are unlike the heretofore known 17-heteroaryl steroids, as in this case, the azole moiety is attached to the steroid nucleus at C-17 via nitrogen of the azole. Most of the compounds are potent inhibitors of both the human and rat CYP17. The most potent compound, 3β-hydroxy-17-(1H-imidazole-1-yl)androst-5,16-diene (VN/85-1), with a Ki value of 1.2 nM is 32 times more potent than ketoconazole (Ki = 38 nM).
Spectroscopic studies with a modified form of human CYP17 indicate that the inhibition process involves binding of steroidal azole nitrogen to the heme iron of the enzyme. In cultures of human prostate cancer cell line (LNCaP), VN/85-1 effectively blocked the growth-stimulating effects of testosterone (T) and dihydrotestosterone (DHT), and was shown to manifest anti-androgenic activity \(^{10}\). In Sprague Dawley male rats, VN/85-1 suppressed T and DHT to basal levels after two 2 weeks of daily dosing at 50 mg.kg\(^{-1}\).day\(^{-1}\) (ref.\(^{11}\)). Furthermore, remarkable antitumor activity was also observed against human prostate cancer (LNCaP) xenografted in combined immunodeficient (SCID) mice\(^{10,12}\). Our most potent inhibitors are currently in development.

In the absence of detailed structural information on the
CYP17 binding site, we have employed a ligand-based computational approach, by analyzing a variety of known CYP17 inhibitors, to identify ligand requirements for inhibiting this enzyme. Alignment of common-feature pharmacophore model with training set of CYP17 inhibitors is presented in Fig. 1. The study has provided the first insight into hypothetical binding requirements for steroidaland non-steroidal inhibitors of CYP17 enzyme. The model may be useful in identification of new and potent CYP17 inhibitors.

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STEROIDAL CYCLOBUTANONES,
SYNTHESIS AND TRANSFORMATIONS
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Cyclobutane oximes rearrange under the Beckmann conditions to give γ-lactams.

In steroids, 5α-spiro[cholestan-3,1′-cyclobutane]-3′-one oximes are transformed to spiropyrrolidinones. Stereosppecificity of the process was clearly established. In the case of α-substituted oximes (α-OH, -OR, -NR2 groups for example), when stabilized cations are formed upon C(α) – C(sp2) bond cleavage, abnormal Beckmann rearrangement has been observed. However, the Beckmann rearrangement of α-chlorocyclobutanone oximes has not been reported.

We prepared steroidal cyclobutanones from ketones in sequence of reactions involving cycloaddition of dichloroke-...
SCUTEPARVIN, A NEW NEOCLERODANE DITERPENOID FROM Scutellaria parvula

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The genus Scutellaria, Lamiaceae (Labiatae) family, occurs with some 360 species spread throughout the world. It is rich with neoclerodane diterpenoids, that usually show some heterocyclic functions: epoxides, lactones, hydrofurans groups. Many of these products have a remarkable antifeedant activity against pest insects. The chemistry of the diterpenoids from Scutellaria was recently reviewed.

Continuing our research program on the components of this genus, we investigated Scutellaria parvula Michx., a species originating from North America (Florida to Quebec).

From its aerial parts we isolated a new neoclerodane diterpenoid, scuteparvin (1). Its structure is rather similar to those of ajugarin V, isolated by Kubo from Ajuga remota, the only difference being the occurrence of the trans-cinnamoyloxy group instead of an acetoxy on C-6.

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SYNTHESIS OF 7,16-DIHYDROXYDEHYDRO-EPANDROSTERONE AND DERIVED HAPTENS

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The analysis of polyhydroxylated dehydroepiandrosterone (DHEA) derivatives is important for the improvement of diagnostic methods for the autoimmune diseases and for the further extending of our knowledge about the markers of neoplastic processes. Our project is dealing with rare metabolites, isomers of 7,16-dihydroxy-DHEA (1), and corresponding haptens for radioimmunoassays.

Synthetic routes for this type of steroids started with DHEA, which is transformed initially to a corresponding isomer of 7-hydroxy-DHEA. Stereoselective introduction of 16α-hydroxy group was accomplished using two principal ways. The first approach used formation of suitably substituted enolates from 17-ketones, selective epoxidation of 16,17 double bond, and subsequent rearrangement into 16α-hydroxy-17-oxo moiety. Second method used bromination of 17-ketone into 16α-position and then solvolysis in a mixture of N,N-dimethylformamide – water into 16α-hydroxy derivative. Both these procedures were used also for a preparation of 16α-hydroxy derivative from 7-oxo-DHEA.

Haptens were prepared by a modification of above methods, the 19-(O-carboxymethyl) group being introduced mainly in the first stages of syntheses. Final CMO derivatives will be coupled with bovine serum albumin and simultaneously used for a preparation of respective homologous tracers. This after generation of polyclonal antibodies enables completion of kits for radioimmunoassays.

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SYNTHESIS AND OXIDATION OF TERPENESULFIDES OF BORANE STRUCTURE

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Terpenic sulfides obtained on the base of available natural compounds are of significant interest as perspective biologically active compounds. The development of selective ways of terpenic sulfides oxidation will allow to synthesize chiral terpenic sulfoxides, which can be used as complex formers of different purpose, including chiral ligands, using in enantio-selective reactions. We have carried out the synthesis of diborneyl sulfide and dibornyl sulfide by the following scheme 1.

The structure of terpenic sulfides is confirmed by the methods of NMR-spectroscopy.

Earlier we shown high hemoselectivity of symmetrical and asymmetrical dialkyl-, alkylaryl-, diaryl-, dihalogendiaryl-, dibenzyl sulfides oxidation by chlorine dioxide. The study of chlorine dioxide reaction ability in the reactions of synthesized terpenic sulfides oxidation is carried out in the present work. The reaction products are isolated and their structure is established by the methods of IR and NMR-spectroscopy.

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and 2 % human urine\(^8\). Parasites were harvested from the medium on day 4, in which the high percentage of infective forms (metacyclic promastigotes) was found. After being harvested from the medium, parasites were counted in Neubauer’s chamber and adjusted to a concentration of 4◊10\(^6\)promastigotes/ml using the supernatant of both cultures as diluents. The substances were added, in different concentrations and were dissolved in small amounts of DMSO. For the larvicidal bioassay, 4th instar larvae of \textit{Aedes aegypti} were used and the test followed the recommendations of WHO\(^9\). BSLA was conducted as recommended\(^10\).

The results of the bioassays are presented in Table I. IC\(_{50}\) values indicate the effective concentration of a compound in mg.ml\(^{-1}\) necessary to achieve 50 % growth inhibition.

Our data showed that among the lapachol analogues assayed against \textit{L. amazonensis}, all the compounds showed IC\(_{50}\) lower than 10 µg.ml\(^{-1}\) and the most effective was the acetylisolapachol (IC\(_{50}/24\) h = 1.6±0.0 µg.ml\(^{-1}\)) (Table I). Isolapachol (2) and acetylisolapachol (5) were also significantly active against \textit{L. braziliensis}, with activities even superior to the reference drug, pentamidine isethionate. In relation to \textit{Artemia salina}, the most active compounds were isolapachol (2) and its derivatives (4 and 5). Concerning lapachol (1), the salt (3) showed a significant decrease on activity in the BSTA. Several salts of lapachol and isolapachol (Na\(^+\), Li\(^+\) and K\(^+\)) were assayed against \textit{Aedes aegypti}. The sodium salt of isolapachol was significantly active, with IC\(_{50}\) = 3.48 µg.ml\(^{-1}\). Despite the absence of the complete set of activities toward \textit{Aedes aegypti}, this result is very stimulating. In general, in all the performed assays, isolapachol and derivatives have been shown to be the most active.

The results so far obtained suggest a continuing search for active compounds within the class of 3-alkyl-2-hydroxynaphthoquinones.

**Acknowledgements to CNPq, RHAE/CNPq, CAPES and FAPEAL.

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**NEW PENTACYCLIC TRITERPENE ESTERS FROM Peltastes peltatus (VELL.) WOODSON**

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\textit{Peltastes peltatus} (VELL.) WOODSON (Apocynaceae) is a creeper widely spread over in the southern states of Brazil.
Species belonging to the genus *Peltastes* are rarely considered in the chemical literature and no report concerning the chemistry is available. The mutagenic activity of the aqueous extract of *P. peltatus* has been recently reported. The aim of the present work was to investigate the chemical composition of *P. peltatus*. Plant material was collected in Alagoas state (Brazil) and voucher specimen (number 3268) has been de- posited in the herbarium of the Department of Botany of the University of Brasília.

The air-dried and powdered stem (2.7 kg) of *P. peltatus* was extracted in a Soxhlet apparatus with EtOH (10 L) to yield 185 g of crude extract. After suspension in MeOH/H₂O (3:2) solution and extraction with EtOH (10 L) to yield an amorphous material (0.8 g). The structures of these compounds were established by MS, 1D- and 2D-NMR experiments as well as by chemical degradation.

The molecular formula of compound 1 was assigned as C_{41}H_{58}O_{2} based on elemental analysis, 1H and 13C NMR and EIMS (M⁺, m/z 582). Its IR spectrum showed absorption bands at 1708 and 1242 cm⁻¹, while its UV spectrum showed absorption maxima at 247 and 308 nm (log ε = 4.39; 4.48, MeOH), related to an aromatic system conjugated with a diene (König and Rimpler, 1985). The 1H NMR spectrum of 1 revealed the presence of eight tertiary methyl groups (δₚ 0.83, 0.87, 0.88, 0.91, 0.93, 0.98, 0.99 and 1.14) and one olefinic hydrogen (δₚ 5.18, br, J = 3.4 Hz), typical of β-amyrin. This was also supported by the observation of a base ion peak at m/z 218 corresponding to the ion resultant from the reverse Diels–Alder fragmentation characteristic of the derivatives of Δ^{12}-oleanene/ursene.

The 13C NMR spectrum of 1 showed only 39 carbon signals (two sets of carbon signals being superimposed), thirty of them corresponding to the triterpenoid moiety and the remainder compatible with a phenylpenta-2,4-dienoyl moiety comparable to that described in an iridoid from *Avicennia marina*. The ester bonding at C-3 was further confirmed in an HMBC experiment. The relative stereochemistry of the diene was assigned on the basis of coupling constants measured in 1H-NMR and on results obtained from a NOESY experiment. Hydrolysis of 1 furnished a triterpenoid alcohol identified as β-amyrin by comparison with reported spectral data. The acid obtained from the hydrolysate was identified as 5-phenyl-(2,4E)-penta-2,4-dienoic acid.

Peltastine A (2) was isolated and characterized as a binary mixture in a ratio of 1:2 with β-amyrin 5-phenylpenta-2,4-dienoate (1). Its structure was determined mainly from comparison of the 1H and 13C NMR spectral data and mass spectrum of mixture with those of compound 1. Alkaline hydrolysis of the mixture (1+2) furnished the 5-phenyl-(2,4E)-penta-2,4-dienoic acid (4) along with the mixture of α- and β-amyrins.

Peltastine B (3) was isolated as a white amorphous solid, m.p. 170–172 °C (EtOH). The 1H and 13C NMR spectra of 3 were closely related to those of compound 1, except for the triterpenoid moiety. Its molecular formula C_{16}H_{20}O_{2} was established by EIMS (M⁺ m/z = 582) in combination with 1H and 13C NMR, indicating that 3 was isomeric with compounds 1 and 2. The presence of an ester linkage in 3 was indicated by the carboxyl signal at δₚ 167.1 together with IR absorption at 1709 cm⁻¹.

The 13C NMR spectrum of 3 showed similar carbon shifts as lupeol acetate and lupeol cinnamate for the lupene part of the molecule. Alkaline hydrolysis of this compound furnished 5-phenyl-(2,4E)-penta-2,4-dienoic acid (4) and the lupeol, identified by comparison with reported data.

To our knowledge, peltastines A (2) and B (3), and β-amyrin juarezate (1) are the only representatives of triterpene esters bearing a conjugated phenyldiene moiety. The 5-phenyl-(2,4E)-penta-2,4-dienoic acid is not usual as natural product, but it has been found in the form of a triterpenoid ester in *Marsdenia pringlei* and in iridoid esters in *Avicennia marina*.

The authors would like to thank Professor José Elias de Paula for collecting and identifying the plant material, CNPq, CAPES and FAPEAL for financial support and Centro Nordestino de Aplicação e Uso de RMN da Universidade Federal do Ceará (CENAUREMN, Fortaleza, CE, Brazil) for NMR spectra.
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CARVONE AS A STARTING MATERIAL FOR THE TOTAL SYNTHESIS OF STEROIDS

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Carvone is a natural product which can be isolated from caraway seeds (S-(+)-carvone) or from mint (R-(+)-carvone). In our lab, these compounds have been applied as a starting material for the synthesis of several more complex natural products of agricultural or medical relevance, as well as fragrance compounds. Preliminary research has also demonstrated its usefulness as a starting material for the synthesis of steroids. Two main pathways are therefore under investigation using carvone either as ring D or as ring B of (homo)steroid skeletons.

A route toward C,D-cis coupled steroid skeletons, in which the use of carvone leads to an enantiomERICALLY pure D-homo- steroid skeleton, has already been developed and has been accepted for publication.

Mukaiyama reactions play an important role in our chemistry and we have been able to show that a Michael-Mukaiyama domino reaction sequence can lead to tricyclic systems which, using appropriately functionalised starting materials, could be further converted into steroid-like compounds. This chemistry is currently under investigation.

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THE PREPARATION AND MIGRATION OF DOUBLE BONDS IN 22(17→28)-ABEO LUPANE DERIVATIVES

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Triterpenoids, especially lupane derivatives are subject of intensive biological studies in the last decade because of their anti-cancer and anti-HIV activities. Among biologically active triterpenoids are commonly present lupane derivatives...
bearing more oxygen containing functional groups or degra-
deterpenoids.

Anhydrobetulinines, 22(17→28)abeo lupane derivatives, were
studied as possible intermediates for the preparation of lupane
derivatives with more degraded skeleton. Migration of double
bonds in the solution of hydrogen bromide in acetic acid led
to the mixture of many dienes with double bonds on rings C,
D and E and two unexpected products: Compound with ace-
tylated aromatised ring E and spirocyclic compound. Forma-
tion of these two compounds is discussed.

Structures, configuration and conformation of all com-
ponents prepared were studied mainly using correlation NMR
techniques.

CONSTITUENTS AND BIOLOGICAL ACTIVITY

OF THE ESSENTIAL OILS FROM Sideritis italic a
(Miller) Greuter et Burdet (Lamiaceae)

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The genus Sideritis (Lamiaceae) counts at least 150 spe-
cies occurring in temperate and tropical regions of the north-
ern hemisphere; the countries around the Mediterranean Sea
are particularly rich. Several species are widely used in the
folk medicine of many countries for their anti-inflammatory,
antispasmodic, carminative, sedative, antitussive, stomachic,
anticonvulsant and antifeedant activities. The essential oils are
used for many therapeutic purposes, for instance as pulmonary
disinfectants, diuretics, stomachics, neurorelaxants. The aeri-
parts of Sideritis taxa have been largely investigated and
diterpenoids were the main metabolites isolated from them. On
the other hand, many papers on the composition of the essen-
tial oils have been published and antimicrobial and
algætic activities have been pointed out. The species Sideri-
tis italic a occurs in Sicily and in Southern Italy and previous
papers reported the occurrence of several ent-kaurane
terpenoids in the aerial parts. The present is the first paper on
the chemical composition of the essential oil from leaves
and flower heads of this plant. Oils were extracted and exami-
ned following the usual procedure. The yields of the essential
oils from flower heads and leaves of S. italic a were 0.16 %
and 0.14 %, respectively. 49 compounds in the flower heads
and 31 compounds in the leaves, amounting to 93.7 % and
92.4 % of the essential oil, were identified. In the oil from
flower heads kaur-15-ene and β-cubebene were the main
components representing one third of the oil. p-Methoxyace-
tophenone was the major component of the oil from leaves.

The results of the studies on the allelopathic activity of the oils
put in evidence an inhibiting action, in vitro, as on the percen-
tage of germination of the seeds as on the development imme-
diately following to the germination of the seed (to the light)
of Raphanus sativus.

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BIOSYNTHESIS OF 2,3-EPOXYBRASSINO-
STEROIDS IN RYE SEEDLINGS

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2,3-Epoxybrassinosteroids such as secasterone and 24-
epi-secasterol previously have been reported in seeds of
Secale cereale and Lycinis viscara, respectively. We ana-
lyzed seedlings of two rye varieties (Secale cereale, cv. “So-
rom” and cv. “Petka”) for the occurrence of brassinosteroids
with special emphasis on 2,3-epoxybrassinosteroids and puta-
tive biosynthetic precursors. Secasterone and 2,3-diepi-seca-
sterone, which is reported in this study for the first time as
a naturally occurring compound, were found in rye seedlings.
In addition, secasterol, the first 2,3-olefinic brassinosteroid
has been identified from the same plant source. Secasterone
and 2,3-diepi-secasterone were synthesized as deuterated ana-
lytical standards and, deuterated teasterone, typhasterol, and
secasterol were synthesized as putative biosynthetic precu-
sors. Feeding experiments using deuterated intermediates were
conducted in vitro, were carried out in rye seedlings (Secale cereale) in order to
investigate biosynthetic incorporation into 2,3-epoxybrassino-
steroids. Biosynthetic products were identified by GC-MS-
-SIM analysis.

Deuterated teasterone, and typhasterol, upon administra-
tion to rye seedlings, were converted to secasterol, secastero-
e and 2,3-diepi-secasterone. Additional feeding experiments
showed a high conversion rate of secasterol to both 2,3-eponyx-
brassinosteroids. From these findings, the biosynthetic se-
Brassinolide I is known as the most potent compound for plant growth promoting activity\textsuperscript{1}. It was reported\textsuperscript{2} that brassinolide analog II showed even better activity under the field conditions, although it was completely inactive under the rice lamina inclination test. In our structure-activity relationships studies of brassinosteroids\textsuperscript{3} we prepared brassinolide analog VI, which shows better activity already in the rice lamina inclination test in comparison with brassinosteroid II.
Synthesis of our brassinosteroids started from (22E)-5α-ergosta-2,22-dien-6-one (III), which was carefully hydroxylated by osmium tetroxide for 2 hours and the acquired (22E)-2α,3α-dihydroxy-5α-ergosta-2,22-dien-6-one (IV) was transformed to its acetonide (V). Subsequent epoxidation with 3-chloro-perbenzoic acid gave compound VI.

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DIBAL REDUCTION OF SOME STEROIDAL LACTONES

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In the course of our work on allopregnanolone analogues, we prepared derivatives of 2- and 4-oxa-5α-pregnane-3,20-diones 1 and 2 and studied their reduction with DIBAL. The lactone 1 was prepared from 5α-pregn-1-ene-3,20-dione by treatment with lead tetraacetate in aqueous acetic acid and the resulting crude secocadahyde-acid was reduced with sodium borohydride. The starting material for the synthesis of the lactone 2 was progesterone: One-step oxidation of progesterone by means of peroxydisulfuric acid in glacial acetic acid was employed; the reagent was prepared from potassium persulfate and concentrated sulfuric acid. The lactones 1 and 2 were reduced with DIBAL in toluene at −78 °C (ref. 3). Biological activity of some products will be reported.

This work was supported by grants No. S5011007 and Z 4055905.

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ENANTIOSELECTIVE SYNTHESIS OF (2R,6R)-2,6,10-TRIMETHYLUNDECANE-1-OL – CHIRAL PRECURSOR FOR THE PREPARATION OF NATURAL VITAMIN E

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The condensation of (S)-chromanyl ethanol 2 (synthesized previously by us) and (R,R)-phosphorane 3 is the key step of one of the approaches to the synthesis of the natural vitamin E – (2R,4S,8R)-α-tocopherol (1). (R,R)-phosphorane 3 is obtained from (2R,6R)-2,6,10-trimethylundecan-1-ol (11).

We developed the effective enantiospecific synthesis of alcohol 11 based on the dehydration of (3R,7R,11R)-isophy-
tol (5) obtained via the ozonolysis of chlorophyll (4) followed by the interaction of \( R, R \)-phytene with vinylmagnesiumbromide. It was found, that the reaction of \( \text{TsOH-SiO}_2 \) with isophytol 5 under the mild conditions resulted in the mixture of phytadiens 6 (a mixture of \( E/Z \)-isomers 1:3) and 7 in a ratio 6:1 in a quantitative yield. Ozonolysis of the diene mixture 6, 7 and the further oxidation of the ozonolysis products with \( \text{KMnO}_4 \) led to the easily separated mixture of \( (4\text{R}, 8\text{R})-4,8,12\)-trimethyltridecane acid 8 and phytene (9). The oxidative decarboxylation of the acid 8 yielded \( (3\text{R}, 7\text{R})-3,7,11\)-dodec-1-ene (10), which ozonolysis followed by the reaction with \( \text{NaBH}_4 \) gave the target alcohol 11.

REFERENCES

STABILITY OF 7-OH EPIMERS OF 3\( \beta \) -HYDROXYANDROST-5-EN-17-ONE (DHEA)

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7-Hydroxylation of 3\( \beta \)-hydroxyandrost-5-ene steroids is a reaction occurring in many mammalian tissues. Hydroxylation of 3\( \beta \)-hydroxyandrost-5-en-17-one (DHEA) with rat liver homogenate at 7\( \alpha \)-position and subsequent conversion to other 7-oxygenated steroids in the sequence DHEA →
7α-hydroxy-DHEA → 7-oxo-DHEA → 7β-hydroxy-DHEA was described. These 7α-OH and 7β-OH isomers have beneficial biological effects (e.g. on immune system) but their activity is different. Therefore, it seems to be useful to prove the stability of both isomers under different conditions.

We have found that both 7α-OH and 7β-OH isomers are unstable under acid conditions, they isomerize to each other. However, in alkaline solution were both isomers stable.

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SIMPLE BRASSINOLIDE ANALOGUES EXHIBITING TYPICAL BRASSINOLIDE ACTIVITY
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A new synthetic brassinolide analogue, 2α,3α-dihydroxy-17β-(3-methylbutyryloxy)-7-oxa-7α-homo-5α-androstane-6-one (11), has been shown to exhibit typical brassinolide activity – splitting of the bean second internode. It was prepared from the known lactone 2α,3α,17β-trihydroxy-7-oxa-7α-homo-5α-androstane-6-one (4) which was transformed to an isopropylidenedioxy derivative. After protection of 2α- and 3α-hydroxy group it afforded the 2α,3α-isopropylidenedioxy-17β-(3-methylbutyryloxy)-7-oxa-7α-homo-5β-androstane-6-one (7) on treating with 3-methylbutyryl chloride in pyridine. The analogue with a 2-methylbutyric moiety (10, 2α,3α-dihydroxy-17β-(2-methylbutyryloxy)-7-oxa-7α-homo-5α-androstane-6-one) in position 17β stimulated only twisting of the bean second internode.

However, in the second bean internode bioassay 100 times more 10 or 11 compared to 24-epibrassinolide is required to obtain the same effects. Analogues with β-oriented hydroxyl groups at C-2 and C-3 (14, 15), a 6-ketone (17, 18) or 6-oxa-7-oxa-lactone system (12, 13), in ring B lack the typical brassinolide activity. In addition, the active brassinosteroids applied to the second internode stimulated a similar, but 30 % lower elongation of the first internode. From data presented here we conclude that the presence of two hydroxy groups in...
the positions 22 and 23 of the brassinolide side chain, which are considered as a key structural requirement, is not absolutely necessary for a compound to exhibit typical brassinosteroid activity. Nevertheless, these compounds have generally 2–10 times lower activity than that having 22,23-vicinal diol in the side chain.

**IMMUNOANALYTICAL SYSTEM FOR DETERMINATION OF BRASSINOSTEROIDS IN PLANT TISSUES**

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Brassinosteroids (BRs) are widely distributed natural products that promote growth and passes all properties necessary for classification as a plant hormone. BRs are group of polyhydroxy steroids causing cell elongation, cell expansion, retard leaf abscission, enhance resistance to stress and promote xylem differentiation. BRs have been detected and isolated from seeds, fruits, leaves, galls and pollen.

We have developed polyclonal and monoclonal antibodies against one of brassinosteroids, 24-epicastasterone. Antiserum against this substance was produced by immunizing rabbits and mice with 24-epicastasterone, carboxymethylxilime conjugated with bovine serum albumin (BSA). The conjugates were prepared by mixed anhydrides method. Polyclonal antibodies were purified from rabbit serum by ammonium sulfate precipitation or by affinity purification on protein A columns. The obtained antibodies were tested in enzyme-linked immunosorbent assay (ELISA) using 24-epicastasterone-carboxymethylxilime-peroxidase conjugate. The cross-reactivity of the antibodies was defined on the bases of competitive studies with other brassinosteroids.

We suppose to use the selected broad-specific antibodies in immunoaffinity chromatography (IAC). IAC-HPLC-MS or IAC-HPLC-ELISA system will be used for analysis of endogenous brassinosteroids in plant tissues.

**NEO-CLERODANE DITERPENES FROM Ajuga remota BENTIL (LABIATAE)**

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Ajuga remota is a shrub growing widely in East Africa, and has been used in traditional medicine for the treatment of skin diseases, toothache, headache, fever, dysentery, high blood pressure, stomachache, malaria, edema, pneumonia and liver problems. Results of tests for antifungal and antimarial activities have been reported recently. Following the observation that African armyworms did not attack A. remota leaves, clerodane diterpenes (ajugarin I, II, and III) were isolated as moderately strong antifeedants against Spodoptera exempta (AJG-I and II min conc 100 ppm) and S. littoralis (AJG-I and II min conc 300 ppm). Ajugarin IV was later isolated in trace amount, exhibiting insecticidal activity against Bombyx mori (at 500 ppm, ED90) and growth inhibitory activity against pink bollworm Pectinophora gossypiella (at 500 ppm, ED90). Ajugarin V, an additional new diterpene isolated in trace amount, exhibited neither antifeedant nor insecticidal activity. The isolation of clerodin (no data) as an antifeedant was also reported. The whole plant A. bracteosa (syn. A. remota) afforded recently bracteoin A, 14,15-dihydroajugapitin, and 14-hydro-15-hydroxyajugapitin.

To test the application of HPLC for the effective and rapid determination and quantification of neo-clerodane diterpenes, an extract from aerial parts of A. remota was prepared and examined (C18 column; UV detection; water–methanol gradient elution). The method allowed the quantification of ajugarins I, II, IV, V in the extract of A. remota leaves, and suggest a higher amount of AJG-I than reported previously. Peak area ratios are close to isolated weights of other AJG, and thus, the analytical method can be used to quantify AJG-like neo-clerodane diterpenes.

Furthermore, it was also found that other prospective neo-clerodane compounds were present. The suitability of reversed-phase HPLC for the semi-preparative fractionation of extracts from A. remota was then explored. The presence and reversed-phase chromatographic behavior of dihydroclerodin, ajugapitin, dihydroajugapitin, and the hemiacetalic 14-hydro-15-hydroxy-ajugapitin and clerodin in A. remota was also established. The structures of the known AJG-I, II, IV, V (rt 21, 28, 40 and 42 min in HPLC semiprep conditions) and the newly isolated compounds were established from NMR data.

Dihydroajugapitin (reported as a natural compound in A. parviflora), a newly isolated compound from A. remota, was eluted at 31 min rt. The presence of a hexahydrofurufuran moiety in the molecule was derived from the chemical shifts in 1H NMR [δ 4.07 (dd, J = 11.5, 5.5 Hz), 2.83 (m), 3.83 (2H, m), 5.61 ppm (d, J = 5.0 Hz); assigned to H-11, H-13, H-15 and H-16], as well as in 13C NMR [δ 85.2, 33.4, 42.1, 32.7, 68.3 and 107.7]. Clerodin eluted at rt 36 min, and ajugapitin was also isolated for the first time from A. remota at 37 min. Dihydroajugapitin (reported previously in A. chamaepitys, A. pseudoiva, A. iva, and recently in A. bracteosa) was eluted at 34 min rt.

In fractions eluting at 23–24 min the epimeric mixture of 15-hydroxydihydroclerodin (scutecyprol A) was also isolated. The corresponding mixture of 15-hydroxydihydroajugapitin was also present but in trace amounts at 24–25 min. 15-Hydroxydihydroclerodanes have been reported mostly from the genus Scutellaria.

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The insect antifeedant property of clerodane diterpenes is the most extensively studied bioactivity of these compounds. Various genera of the plant family Labiatae, namely Scutellaria and Ajuga are reported to produce some of the most potent clerodane antifeedants. In Scutellaria, jodrellin B (occurring in S. albida, S. galericulata, S. grossa, S. polyodon, and S. woronowii) and scutecyprol B (found in S. columnae, S. cy-
Isoprenoids are the largest group of secondary metabolites. They share the same five carbon building block isoprene which is derived from isopentenyl diphosphate (IPP). This common precursor can be biosynthesized either via the mevalonic acid pathway (MVA) or via the methylerythritol phosphate pathway (MEP)\(^1,2\). Both MEP and MVA pathways are involved in the formation of isoprenoids of higher and lower plants.

In the course of our studies on the origin of the isoprene building blocks of various plant isoprenoids\(^3,4\), we investigated the sesquiterpene lactone cnicin in axenic cultures of the Astereaceae *Cnicus benedictus*. Cnicin is an ester of a germacrene type sesquiterpene lactone unit with an isoprene like hydroxyethylacrylic acid side chain.

The biosynthetic origin of these two moieties has been elucidated by incorporation of \([1-\text{13C}]\)glucose, \([U-\text{13C}]\)glucose and \([U-\text{13C}]\)isoleucine into cnicin and subsequent quantitative \(\text{\textsuperscript{13}C}\)NMR spectroscopic analysis of the labelled compounds.

The \([1-\text{13C}]\)glucose experiment demonstrated that the sesquiterpene unit is biosynthesized exclusively via the MVA pathway (Fig. 1).

The analysis of the labelling patterns after \([U-\text{13C}]\)glucose incorporation indicated the cyclization of farnesyl diphosphate to germacrene A as a hypothetical intermediate of the sesquiterpene moiety (Fig. 2).

The \([U-\text{13C}]\)isoleucine experiment showed that, despite its isoprene like skeleton, the hydroxyethylacrylic acid side chain is derived from the amino acid isoleucine (Fig. 3). However, qualitative and quantitative labelling patterns of the side chain after \([1-\text{13C}]\)glucose and \([U-\text{13C}]\)glucose incorporations suggest a formation of isoleucine via a non typical biosynthetic pathway\(^5\).

REFERENCES

2-(1-NAPHTYL)-2-PHENYLACETIC ACID IN DETERMINATION OF ABSOLUTE CONFIGURATION OF CHIRAL SECONDARY ALCOHOLS BY $^1$H NMR

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Mosher’s acid (MTPA) has been one of the most favourite chiral derivatizing agents based on acetic acid pattern whose power lies in the anisotropic effect of an arylate. It has already proven its usefulness in the determination of the absolute configuration of chiral alcohols and amines. A new compound exploiting the aromatic anisotropic effect, 2-(1-naphthyl)-2-phenylacetic acid (1-NPA), was designed. A strong enhancement in isochrony $\Delta \delta$ (the difference of chemical shifts of diastereomeric protons) caused by the present naphthyl ring was expected.

Enantiomerically pure acid was obtained from the racemic one by resolution of diastereomeric mixture of (R)- and (S)-1-NPA ($\alpha$)-menthylesters and subsequent hydrolysis. A set of 11 chiral alcohols was prepared with both racemic and enantiomerically pure acid. The $\Delta \delta$ values were tabulated and their absolute values were compared with those of MTPA. Afterwards, a correlation of the $\Delta \delta$ values with the structure of the alcohol moiety revealed that the spectral behaviour followed systematic rules reflecting their steric arrangement. X-Ray structure of (\text{\textalpha{}})-menthylester of 1-NPA was obtained showing phenyl group facing the alcohol moiety rather than the naphthyl unlike the expectations. This may explain why smaller values of $\Delta \delta$ than presumed were observed, although they exceed those of MTPA.

On the other hand, the anisotropic effect in distant atoms was also observed, as citronellol and similar terpenoids were esterified with 1-NPA. The more detailed conformation analysis will be given.

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BRASSINOSTEROIDS: TRYING TO FIND A BETTER WAY TO SELECT THE ACTIVE CONFORMATION TO ESTABLISH A QSAR

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In the field of brassinosteroids (BRs), we have developed a quantitative structure-activity relationship (QSAR) study that provide defined information about the minimum structural requirements necessary to elicit activity as plant growth promoters.

Since the structure of the receptor is not known, indirect methodologies for QSAR studies are required. These methods assume that all information needed to explain the activity is on the structure of BRs. Even BRs can adopt different conformations, due to the presence of flexible points, the active BRs should have a single defined three-dimensional (3D) “active conformation” able to bind to the receptor. On this “active conformation”, atoms involved in binding with receptor ought to have the same spatial situation in all active molecules. Thus, the more complementary is the “active conformation” of a defined BR to the 3D structure of receptor, the most active it should be.

One should take into account that the so-called “active conformation” is not necessarily the preferred conformation adopted in the steroid-receptor complex. Nevertheless, and due to the fact that the structure of the receptor is unknown at the moment, the information gained by comparing the “active conformation” of all the active BRs will be useful in defining a good QSAR. Moreover, one can assume that the energy needed for the “active conformation” of various BRs to adopt the preferred conformation will not differ too much for one active BR to another.

In our QSAR studies the “active conformation” of BRs was selected based on the methods of active analog approach (AAA) using geometrical descriptors as a selection criteria. This approach consists on the selection of the “active conformation” for a reference compound (brassinolide) by comparing all its possible conformers with all the possible conformers of each of the other active BRs. Thus the “active conformation” of brassinolide will be that which results in highest
similarly. Once defined the “active conformation” for brassinolide, this is taken as reference for further comparison with all conformers of a defined BR. The “active conformation” of each BR will be the most similar to the “active conformation” of brassinolide.

According to these geometries very good QSAR models were obtained. Based on GRID methodology we have found a good correlation between the activity and the areas with high probability of H-bonding with receptor. The results obtained until now, suggested that the region near to 23R-OR was more important for eliciting activity than those near to 22R-OR.

Recently, the activity of some of the new compounds obtained in our group don’t fully agree with the contribution on activity, above suggested, for the hydroxyls at C22 and C23 (ref.3). Considering that the “active conformation” for 22S, 23S-BR differs considerably from those of 22R,23R-BR’s we suggested a re-evaluation of the methodology used to select the “active conformation”. In this communication we will present another way to select the “active conformation” based on activity prediction criteria which will allow us to consider the side chain 3D geometry from another point of view. Both methodologies will be compared and discussed.

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3. See poster Iban Jové et al.

BRASSINOSTEROIDS AND WATER STRESS IN RAPE (Brassica napus L.)

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One of the most serious environmental stresses is drought. Not only survival during drought stress but also recovery of stressed plant is crucial for the next plant growth. Synthetic analogues of the brassinosteroids represent new plant growth regulators with the activity to increase plant growth but also to enhance resistance of plants to stress conditions. It was described that 24-epibrassinolide increases root activity, plant growth and weight of the roots and shoots if applied as foliar spray to plants grown after water stress.

One of the first reactions of plants to stress is the ethylene production. The production of ethylene is increase by flooding, high temperature, toxic materials etc. Another phytohormone increase in water stress is abscisic acid in the leaves and roots.

In our experiments the plants of rape were used. The plants were exposed to drought and flooding stresses. These plants were treated with new synthetic brassinosteroid analogues and their influence on the suppression of water stress was studied. The first variant was treated with brassinosteroids 7 days before the beginning of stress as foliar sprays, the second variant was sprayed 5 days after the beginning of stress. Two variants were used as controls: the first one without stress and without any treatment, the second one with stress without any treatment. The influence of stress in all variants was determined by dry means of weight changes, ethylene production, fluctuation of abscisic acid and chlorophyll a and b content.

The dry weight were increased in both type stresses of plants (drought and flooding) treated with 24-epibrassinolide as well as with brassinosteroid analogues. The production of ethylene was not influenced significantly after treatment of neither analogues nor 24-epibrassinolide.

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THE CHIRAL DERIVATIZATION WITH MOSHER’S ACID ACYLSOCYANATE

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Trichloroacetyl isocyanate (TAI) has alredy been proven as a valuable derivatization tool for NMR investigation. Its chiral derivative, (S)-2-chloro-2-fluoroethanolyl isocyanate besides conserving good reactivity of TAI, brings additional informations with respect to the chirality of compound investigated, thus demonstrating the possible role of chiral acylisocyanates in the analysis of optically pure compounds. For obtaining good results with those types of substances, however, the presence of some functional groups is essential. Namely, halogen atom(s) as well as aromatic ring in the molecule significantly accelerates the reaction and positively affects the spectral properties, respectively. Since the Mosher’s acid contains both the trifluoro- and phenyl groups, it seemed...
to be a good starting material for preparing thus derived acylisocyanate.

The preparation of 3,3,3-trifluoro-2-methoxy-2-phenylpropanoylisocyanate (1) follows the easy two step reaction leading from (R)- or (S)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA) to 3,3,3-trifluoro-2-methoxy-2-phenylpropanamide (2). Finally, amide 2 with oxalyl chloride under the controlled conditions affords acylisocyanate 1 pure enough for in situ reaction with chiral alcohols. The reaction with alcohols is usually very fast (without any catalysis) giving mixture of diastereomeric carbamates esters detectable by means of NMR. The analysis of NMR data of database of prepared diastereomers revealed that 1H, 19F and 13C-NMR shifts well correlating with the compound’s structure, so the derivatization may be used only for the optical purity determination, but also for estimation of absolute and/or relative configuration.

Last but not least, results in the form of the characteristic chemical shifts have been compared with results of previous works where was used MTPA³ and α-methoxy-α-(trifluoro- methyl)benzyl isocyanate⁴ as the chiral derivatization reagents.

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SYNTHESIS OF STERIOD CONTAINING MACROCYCLES VIA MULTI COMPONENT REACTIONS

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The synthesis of molecules which can recognize and bind others or catalyze transformations of the bound molecules like an artificial enzyme is one of the most exciting fields in synthetic chemistry. Our aim are molecules with well-defined geometries in which conformational freedom is kept under close control. This criterion can be met by designs based on rigid frameworks combined with elements of controlled flexibility. At the same time, certain dimensions are required and have to be build up fast and efficiently.

With this background we started a program for the synthesis of macrocycles with steroid containing rigid parts using the Ugi-multi component reaction (U-MCR³). Bile acids are the most valuable group of steroids due to their relatively rigid moiety, umbrella shape, chemically different hydroxyl groups, enantiomeric purity, availability, and low cost.

First step for the synthesis of cyclopeptide steroids is the bifunctionalization of bile acids as starting compounds. Good results were obtained with the U-4CR-condensation of the diamine⁵ and disiocyanide synthesized from lithocholic acid, together with isobutyraldehyde and acetic acid (Scheme). After chromatography and preparative HPLC-separation eight
PRODUCTS WERE OBTAINED HAVING THE MOLECULAR ION MASS OF 969 IN THE ESI-MS WHICH SHOWS THE SUCCESSFUL CYCLIZATION. THESE ARE THE FOUR POSSIBLE DIASTEREOMERS OF THE HEAD-HEAD AND HEAD-TAIL REGIOISOMERS. WHEN PARAFORMALDEHYDE WAS USED FOR THE SAME U-4CR ONLY TWO MAIN PRODUCTS WERE FORMED. MOLECULAR MODELLING INVESTIGATIONS SHOWED THAT THE HEATS OF FORMATION OF THE HEAD TO HEAD AND HEAD TO TAIL MACROCYCLES IS NEARLY THE SAME, THE PROBABILITY OF THE FORMATION OF BOTH MACROCYCLES IS CLOSE TO EQUAL. THE CAVITIES OF SYNTHESIZED MACROCYCLES ARE LARGE ENOUGH TO ENCAPSULATE SMALL ORGANIC SUBSTRATES, AND MORE DETAILED STUDIES IN THIS RESPECT ARE UNDER WAY.

REFERENCES

NONGENOMIC STEROID ACTION:
FROM MEMBRANES TO HUMAN PHYSIOLOGY
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According to the traditional model steroid hormones bind to intracellular receptors and subsequently modulate transcription and protein synthesis, thus triggering genomic events finally responsible for delayed effects. Based upon similarities in molecular structure, specific receptors for steroids, vitamin D3 derivatives, thyroid hormone, retinoids and a vanity of orphan receptors are considered to represent a superfamily of steroid receptors. In addition, very rapid effects of steroids mainly affecting intracellular signalling have been widely recognized which are clearly incompatible with the genomic model. These rapid, nongenomic steroid actions are likely to be transmitted via specific membrane receptors. Evidences for nongenomic steroid effects and distinct receptors involved are now presented for all steroid groups including related compounds like vitamin D3 and thyroid hormones. The physiological and clinical relevance of these rapid effects is still largely unclear, but their existence in vivo has been clearly shown in various settings including human studies. Drugs that specifically affect nongenomic steroid action may find applications in various clinical areas such as cardiovascular and central nervous disorders, electrolyte homeostasis and infertility. In addition to a short description of genomic steroid action, this review pays particular attention to the current knowledge and important results on the mechanisms of nongenomic steroid action. The modes of action are discussed in relation to their potential physiological or pathophysiological relevance and with regard to a cross-talk between genomic and non-genomic responses.

Prominent examples of nongenomic steroid action are rapid aldosterone effects in lymphocytes and vascular smooth muscle cells, vitamin D3 effects in epithelial cells, progestosterone effects in human sperm, neurosteroid action on neuronal structures and vascular effects of estrogens. Mechanisms of action are being studied with regard to signal perception and transduction involved, and for various steroids including aldosterone a patchy sketch of a membrane receptor/second messenger cascade shows up in the mist being not essentially dissimilar to cascades involved in catecholamine or peptide hormone action. Aside nonclassical membrane receptors with a high affinity for a particular steroid, these effects appear to vary depending on phospholipase C, phosphoinositol turnover, intracellular pH and calcium, protein kinase C and tyrosine kinases. The physiological and pathophysiological relevance of these effects is not yet clear, but more and more studies indicate that rapid steroid effects on cardiovascular, central nervous and reproductive functions occur in vivo and seemingly transmit physiological and pathophysiological responses. Future research will have to target the cloning of the first membrane receptor for steroids which should be achieved in near future, and the evaluation of the physiological and clinical relevance of these rapid steroid effects.

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ALKYL GLYCOSIDES WITH BIOLOGICAL ACTIVITY

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This research has been based on juvenoids (insect juvenile hormone mimics), which have been developed in past years. All juvenoids studied belong to the 2-(4-hydroxybenzyl)-cyclohexan-1-ol series. Their glycosides belong among hormonogenic substances (juvenogens) capable of liberating the biologically active juvenoids under the effect of biotic factors (enzymes) or abiotic factors (environmental conditions – pH, UV, humidity or oxygen effect etc.).1,2

The present study reflects our effort in investigating physico-chemical and biological properties of 2-(4-alkoxybenzyl)-cyclohex-1-yl-β-D-glycopyranosides. In principle, the compounds are accessible by several most often used methods of synthesis of alkyl glycosides: Fischer-Helrich method, Koenis-Knorr method or a method through auxiliary trichloroacetimidate formation. We have found that the protected derivatives, 2-(4-alkoxybenzyl)cyclohex-1-yl-2′,3′,4′,6′-tetraco- O-acetyl-β-D-glycopyranosides, are available by the Koenis-Knorr synthesis, using 2,3,4,6-tetra-O-acetyl-β-D-glycopyranobromides as glycosyl donors and heavy metal promo-

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ters to perform the reactions (Scheme 1). Using cadmium carbonate as promoter has resulted in highest yields among a series of screened promoters. Subsequent deprotection of the sugar unit may be performed by different methods, depending on functionalities present in the aliphatic chain R (Scheme 1). Rather convenient method of removing acetyl groups consists in using alkalimetal salts in absolute methanol. If another ester functionality is present in the molecule, in the aliphatic chain R, deacetylation by zinc acetate in absolute ethanol usually represents a convenient method. Our investigation has involved synthesis of selected glucosides and galactosides. Selected biological activity data of several juvenogen examplesubstances on non-related insect pest species will be presented.

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REFERENCES

ROLES OF BRASSINOSTEROID BIOSYNTHESIS IN PEA SEED DEVELOPMENT AND GERMINATION

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Brassinosteroid (BR) is a steroidal plant growth hormone involved in cell elongation/enlargement, cell division and cell differentiation. Major BRs are C28 steroids that are synthesized from campesterol (Fig. 1). Campesterol is hydrogenated to campestanol and then converted to castasterone through either the early or late C-6 oxidation pathway. Castasterone is finally converted to brassinolide. The available evidence suggests that brassinolide and castasterone are both biologically-active.

To investigate the roles of brassinosteroids in seed development and germination, we quantified the endogenous BRs and the transcripts of BR synthesis (LKB, LK, PsDWF4, PsCPD1, PsCPD2, DDWF1, PsD), metabolism (PsBAS1) and receptor (LKA) genes in seeds and seedlings of pea (Pisum sativum L.).

As pea seeds rapidly grow, the levels of brassinolide and castasterone were increased but, in fully-expanded seeds, were decreased drastically, indicating brassinolide and castasterone are important for seed growth. In support of this, the PsD expression was increased in conjugation with the increase of castasterone and brassinolide. 6-Deoxocastasterone was accumulated high in fully expanded seeds but rapidly decreased through desiccation presumably by the action of the PsBAS1 enzyme. The levels of upstream precursors, 6-deoxocastasterone, 6-deoxoasterone, 3-dehydro-6-deoxoasterone and 6-deoxotyphasterol were not changed much from immature to mature stages. 6-Deoxocastasterone was the major brassinosteroid in mature seeds and is likely to be an important storage form. Through seed growth, the LK transcript levels remained constant but those of other genes were fluctuated. In mature seeds, the PsCPD1 gene level increased markedly while the levels of LKB, PsCPD2, DDWF1, PsBAS1 and LKA were considerably decreased although still detectable, suggesting that the mRNAs of these genes may be utilized to generate brassinosteroids when seeds germinate.

In imbibed seeds, neither castasterone nor brassinolide were detected but the transcripts of PsD, DDWF1, PsDWF4, PsBAS1 and LKA were increased. One and three days after the imbibition, castasterone was detected in germinating seeds. In these plantlets, the level of castasterone as well as the PsD expression was high in shoots and roots but low in seeds, indicating that the PsD protein seems to be a key enzyme for seed germination.
Fig. 1. Brassinosteroid biosynthesis and related genes in *Pisum sativum* (Bold arrows indicate major biosynthetic pathways)
SYNTHESIS OF 5,10- AND 13,14-SECOSTEROIDS

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A distinctive feature of steroids is the presence of rigid tetracyclic skeleton. The greater conformational flexibility in these molecules, which can be obtained by cleavage of an internal C-C bond to seco steroids, may lead to new biological properties. Results on the synthesis of 5,10- and 13,14-seco steroids using different approaches (radical oxidation, Grob fragmentation, oxidative cleavage) will be presented.

Radical oxidation of the tertiary alcohol 1 with lead tetraacetate in the presence of iodine gave a set of intermediates 4–7, which are suitable for the preparation of 5,10-seco steroids. The reaction proceeds via the radicals 2 and 3. The latter undergoes either hydrogen abstraction at C-1 to afford the Δ11(10)-olefins 4 and 5, or a transannular hydrogen abstraction at C-4. Reaction of this new radical with iodine then leads to the iodides 6 and 7.

Compounds 4–7 were transformed further into steroids with functional groups characteristic for androgens (e.g. 8). Some fragmentation or intramolecular cyclization products like 9,10 were obtained also.

An alternative approach to 5,10-seco steroids was found by ozonolysis of the Δ5(10)-olefin 11, followed by synthetic transformations of diketone 12. Seco steroids containing two double bonds in the AB-cyclic part (e.g. 13) became available in this way.

Key steps in the preparation of 13,14-seco steroids 17–19 were again found in radical oxidation of the 14α-hydroxy derivative 15, followed by the removal of iodine in 16.

A second route to 13,14-seco steroids was based on the Grob fragmentation of hydroxy tosylate 20. Modifications of the resulting unsaturated ketone included hydride reduction of the 14-carbonyl group and hydroboration-oxidation of Δ13,17.
-double bond, which both proceeded stereoselectively, to provide compound 22 as the only isomer.

The structural elucidation of new types of seco steroids and reaction mechanisms will also be discussed.

SEMISYSTEMATIC NOMENCLATURE OF BRASSINOSTEROIDS

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The assignment of a trivial name to a natural product has the advantage of concentrating all the structural, including stereochemical, information in a single, or at most a few, simple word(s), since it is unique to this compound, but without knowing the compound one cannot advance these information. On the other hand, its systematic name carries all structural and stereochemical information, but is usually too long for being frequently used. The trivial name of a brassinosteroid is either derived from the plant source it was first isolated or detected (brassinolide, from *Brassica napus* L.; castasterone, from *Castanea crenata* Sieb. et Zuck.; dolicholide and dolichosterone, from *Dolichos lablab* Adans.; typhasterol, from *Typha latifolia* G. F. W. Mey.; teasterone, from

*Thea sinensis* L.; secasterone, from *Secale cereale* L.), either obtained by addition of adequate prefix(es) to the name of a previously known brassinosteroid (e.g. 28-homobrassinolide, 28-norcastasterone, 25-methyldolichosterone, 3-epi-2-deoxy-25-methyldolichosterone, etc.). More than 50 known brassinosteroids are 3-oxygenated (22R,23R)-5α-cholestane-22,23-diols, of plant origin, bearing alky or oxy substituents, conjugated or not to sugars or fatty acids. This general structural feature allows the proposition of semisystematic names to the brassinosteroids, in which (22R,23R)-2α,3α,22,23-tetrahydroxy-5α-campestane, of trivial name 6-deoxocastasterone, is considered the functional parent compound and is named brassinostane or brassinane. The closely related compounds of trivial names, 6α-hydroxycastasterone, castasterone and brassinolide, would then be named 6α-brassinostanol, brassinostanone and brassinostanolactone, respectively, or 6α-brassinol, brassinone and brassinolide. The semisystematic names of the other members of the brassinosteroid family shall be given according to the established rules for naming natural products. The use of these semisystematic names would avoid some mistakes in assigning trivial names to the brassinosteroids and the unpractical constant usage of their systematic names.

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