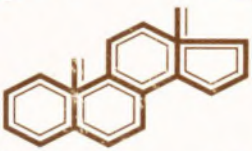
Book of Abstracts

16th CONFERENCE ON ISOPRENOIDS



Prague 1995

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ORAL PRESENTATIONS

STEROID ANTIANDROGENS - NATURAL AND SYNTHETIC

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According to the definition (Dorfman 1970) antiandrogens are compounds which prevent androgens from expressing their activity at target tissues. Their mechanism of action is based on the competition of the substances with androgens for the binding sites on androgen receptor in the target tissue. Some of them, esp. cyproterone acetate, flutamide or cassodex, are sucessfully used in the treatment of benign and malignant prostate diseases in males and for the correction of symptoms of hyperandrogenaemia such as acne, hirsutism, androgenic alopecia and seborhoe in women. A number of synthetic antiandrogenic substances, steroids as well as non-steroids, was prepared until now. One of the compouds tested in praeclinical studies as early as in the sixtieths by Syntex was 17α-methyl-B-nor-testosterone, synthetised first in Prague. The potent androgenic effects of this steroid prompted us to synthetize and to test a series of steroids with modified rings A and/or B. A-nor-, B-nor-, A- or B-homo, A- or B-cyclopropano- and 4,5-seco-steroids were studied. Among more than 80 newly synthetised compounds we could identify several weak androgens and at least ten potential antihormones, e.g. 4,5-cyclopropano-1-androsten-17β-acetoxy-3-one, 4,5-seco-testosterone or 17α-methyl-4,5-seco- testosterone.

One of the tested compounds was epitestosterone, a naturally occurring 17α-epimer of testosterone, which for a long time has been believed to be deprived of any biological activity. We could demonstrate, that epitestosterone not only exhibits the classical antiandrogenic properties, i.e. it competes with testosterone for the binding sites on the androgen receptor, but also inhibits strongly the 5α-reductase, reduces the biosynthesis of testosterone in the testis by inhibiting $C_{17,20}$-lyase and influences in a biphasic mode the production and secretion of LH and to a lesser extent of FSH. Some theoretical considerations as to the role of epitestosterone in the pathogensis of androgen-dependent diseases are at present under investigation.

DIOXIRANES IN THE CHEMISTRY OF STEROIDS

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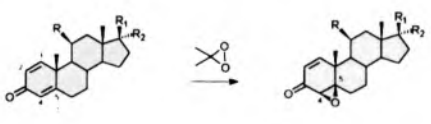
Dioxiranes **1** constitute a new class of versatile oxidants, powerful in their action, yet selective and capable of performing under extremely mild conditions, which allows one to carry out an impressive variety of synthetically useful transformations.[1]

1

R = CH_3 ; CF_3

The target of our project was to study the high reactivity of dioxiranes as epoxidizing and C-H σ bond oxyfunctionalizing agents towards steroids, in order to obtain synthetic key intermediates from readily available compounds.
Good results were obtained, in terms of chemo and stereoselectivity, from the epoxidations of C_5-C_6 double bond in pregnanic and cholestanic structures as well as from that of C_4-C_5 in Δ^{4-5}-3-oxo systems.[2]
The most significant results were on epoxidations of 1,4-dien-3-oxo steroidal systems.[3] Their two double bonds in A-ring are known to be hardly epoxidized with standard procedures. With dimethyldioxirane a good reactivity towards the more nucleophilic 4,5-double bond was noted for compounds **2**, with a high regio and β-stereoselectivity.



2 **3**

R = H, OAc
R_1 = $COCH_2OAc$, C_8H_{17}
R_2 = H, OH
R_1 = R_2 = O

4 **5**

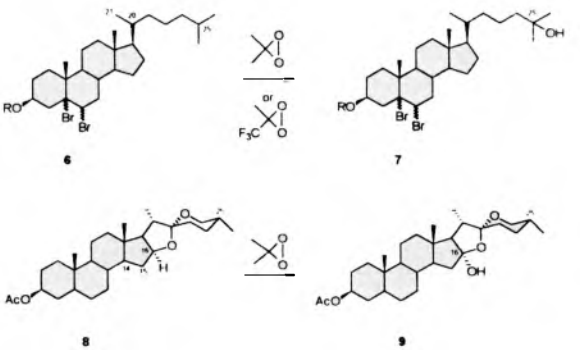
R_1 = $COCH_2OAc$, R_2 = OH
R_1 = R_2 = O

A complete inversion of the regioselectivity of the epoxidation occurred with compounds having also a carbonyl moiety on C_{11}, as in **4**. The main product was

1α,2α-epoxide **5** suggesting a close dipole-dipole interaction between dioxirane dipole and that of carbonyl.

Potential applications in synthesis of these alternative epoxidations will be also discussed.

Important results on the O-insertion on C-H σ bonds were the oxyfunctionalization at C_{25} of 5α-cholestanic derivatives[4] **6,** which allows a more easy access to the 25-hydroxy derivative of Vitamine D_3. A fine control of dioxirane reactivity was pointed out by the selective oxyfunctionalizations at C_{14} of 5α-androstanes, at C_5 of coprostanes[6] and the O-insertion at C_{16}-H moiety of sapogenins[7] **8**.



6 7 8 9

All these oxyfunctionalizations are stereospecific and, therefore, confirm a one step O-insertion mechanism. They support recent studies[8] which propose a preferential approaching trajectory of dioxirane toward the C-H bond. As a result, a great sensitivity of dioxirane to stereoelectronic effects was evident and allowed us to achieve significative synthetic results.

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WESTPHALEN, WESTPHALEN-TYPE AND RELATED SKELETAL REARRANGEMENTS OF STEROIDS

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The Westphalen rearrangement[1], reaction characteristic for 5α-hydroxysteroids, is known to occur in acetic anhydride under specific catalysis of sulfuric acid. The reaction proceeds *via* the steroid acetyl sulfate (R-O-SO_2-OAc, R = steroid rest) which then undergoes 10β-methyl migration and 9α-proton abstraction to give 5β-methyl-19--nor-$\Delta^{9(10)}$-steroids. Usually, the product is accompanied by the isomeric rearranged 5β-methyl-19-nor-$\Delta^{1(10)}$-steroid, 5α-acetoxy derivative of the substrate and the product of simple 1,2-elimination. No rearrangement products were obtained from 6β-methyl--5α-hydroxy-, 6α-substituted-5α-hydroxy- and 5β-hydroxy-steroids under Westphalen rearrangement conditions.

In 1986 we have proved[2] our earlier suggestion[3] that application of $(CF_3CO)_2O$ instead of Ac_2O should increase the yield of the rearranged product. Further studies on the influence of solvent or acid anhydride on the course of the Westphalen-type rearrangement showed that $KHSO_4$-TFAA is indeed the best reagent for this reaction. Moreover, we have succeeded in isolating the intermediate steroid sulfate in the form of its sodium salt (R-O-SO_2-ONa). Reaction of $KHSO_4$-TFAA with 6β-substituted (-NO_2, -N_3, -OAc, -Br, -OMe) 5α-hydroxysteroids gave, with the exception of 6β-CH_3, good or very good yields of the rearrangement products. Similarly behaved C-6 substituted (=O, =N-OAc, =N-NO_2) 5α-hydroxysteroids. However, 6-hydroxyimino-5α--hydroxysteroid underwent qualitatively the Beckmann rearrangement to give the 5,6-seco-5-oxonitrile. No rearrangement products were detected in reactions of 6β-methyl-, 6α-substituted (-NO_2, -OAc) and C-6 unsubstituted 5α-hydroxysteroids.

The extension of this rearrangement to 3β-acetoxy-5-hydroxy-5β-cholestan-6-one resulted in the first successful Westphalen-type rearrangement of 5β-hydroxysteroid. Depending on the solvent used (trifluoroacetic acid or 1,2-dimethoxyethane) the main isolated products were 9(10→5α)-abeo-C(5)-spirans or 1(10→5α)-abeo-C(5)-spirans, respectively. The products were accompanied by small quantities of 5β-methyl-A--homo-B,19-dinor-$\Delta^{9(10)}$-steroid and Δ^4-6-oxosteroid.

Continuing of our studies on skeletal rearrangements of steroids we have also synthesized 5-amino-6-oxo and 5-hydroxylamino-6-oxo analogs. Nitrosation reaction of both 5α-amino-6-oxosteroid and 5α-hydroxylamino-6-oxosteroid in THF-AcOH-H_2O mixture led to isolation of inseparable mixture of 5β-methyl-19-nor-$\Delta^{9(10)}$- and 5β-methyl-19-nor-$\Delta^{1(10)}$-steroid. The minor product of these reactions was Δ^4-6-oxosteroid, resulted from 1,2-elimination reaction. The calculated ratio of

Hofmann ($\Delta^{1(10)}$-olefin) to Saytzeff ($\Delta^{9(10)}$-olefin) products from the nitrosation of aminosteroid and hydroxylaminosteroid was 1:2.2 and 1:1.7, respectively. Similar treatment of the 5β-amino-6-oxosteroid and 5β-hydroxylamino-6-oxosteroid gave exclusively one type of rearrangement product, namely, 9(10→5α)-abeo-C(5)-spirans.

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STEROLS AND VITAMIN D DERIVATIVES. PARTIAL AND TOTAL SYNTHESIS

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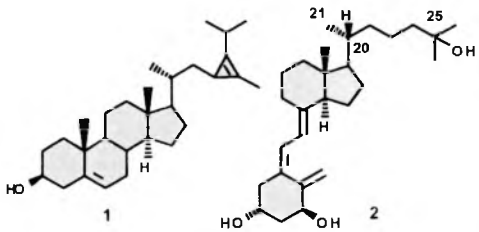
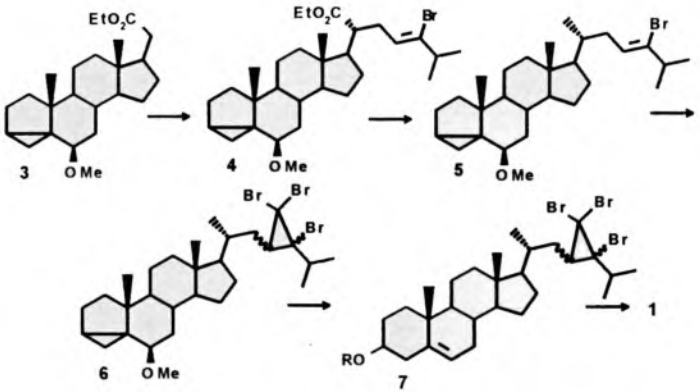


The lecture will focus on partial synthesis of calysterols (**1**) and vitamin D derivatives (as **2**) , and on a new methodology for total synthesis of vitamin D.

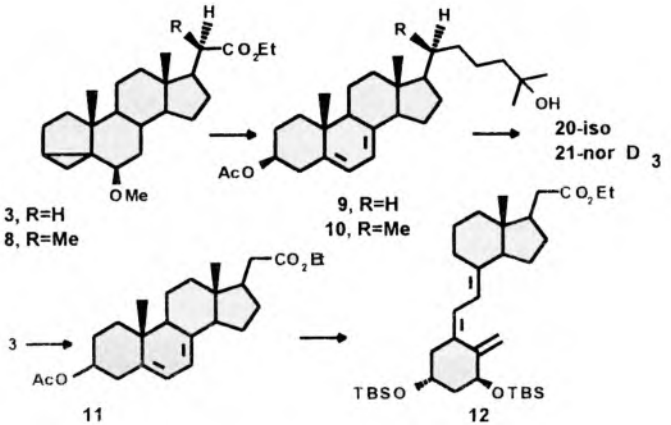
Calysterols have been first isolated from a Mediterranean sponge many years ago.[1] In spite of considerable efforts[2] no synthesis of these compounds has been achieved. Our approach[3] (Scheme 1) involves alkylation of ester **3** with 1,3-dibromo-4-methylpent-3-ene, reduction of the ester group in **4** and cyclopropanation of **5**. The cruciai step consist in debromination and methylation of **7**.

Scheme 1



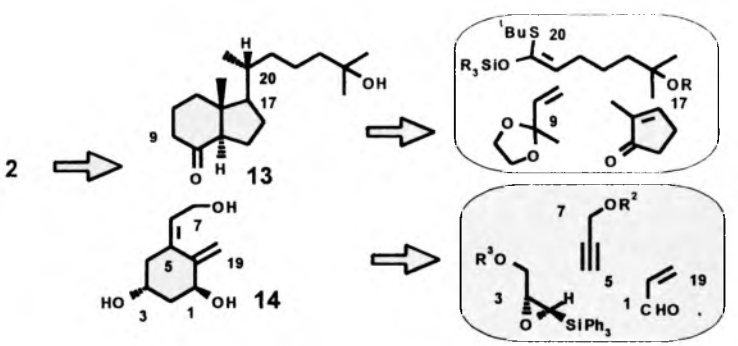
In view of the favourable pharmacological properties some of vitamin D analogues, it is of importance to prepare a variety of vitamin D - related structures modified in the C\D rings and\or in the side chain.[4] The synthesis of 20-iso- and 21-nor vitamin D derivatives is highlighted in Scheme 2. Pregnanoic esters **3** or its methylated derivative **8** were subjected to the extension of the side chain[5] and then transformed into dienes **9** or **10**, respectively. Photolysis followed by standard transformations afforded the required analogues. The synthesis embraces approximately 20 steps and affords the products in ca. 5% yield. 20-Iso- and 21-norvitamin D derivatives show interesting biological properties.[6] In more versatile approach to C_{20}/C_{21} modified analogues vitamin D triene **12** is used as the common precursor.

Scheme 2



An alternative relatively short synthesis of vitamin D will be dicussed.[7] The concept of synthesis is presented in Scheme 3.

Scheme 3



The racemic CD rings side chain fragment (**13**) was constructed from three components in seven steps. Optically active fragment A was prepared from triphenylsilylglycidol, acrolein and propargyl alcohol derivative in ten steps.

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CYCLOADDITION-FRAGMENTATION STRATEGIES IN THE SYNTHESIS OF NEW-GENERATION ESTROGENS

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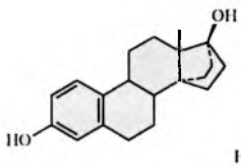
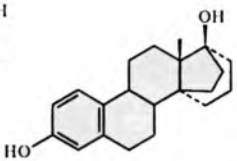
The finding that 14,17-ethanoestra-1,3,5(10)-triene-3,17β-diol **1** is a potent oral estrogen[1] has prompted a systematic investigation into structure-activity relationships of ring D modified analogues of estradiol and estriol. The overall conformational profile of the 14α,17α-ethano analogue **1** closely resembles that of estradiol, but molecular mechanics calculations conducted on structural variants of this parent reveal that changes in the bridge size and disposition introduce subtle deviations in the spatial relationships between the polar groups at C-3 and C-17, and in the steric environment of C-17. In order to test the influence of these deviations upon receptor binding affinities, syntheses of the 14α, 17α- and 14β,17β-propano analogues **2** and **3** of estradiol were undertaken.



1



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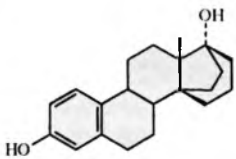

3

Intramolecular aldol condensation of 17β-acetoxy-3-methoxy-20-oxo-19-nor-17α-pregna-1,3,5(10)-triene-14-carbaldehyde [from a cycloaddition-oxidative cleavage sequence on 3-methoxy-16-methylestra-1,3,5(10),14,16-pentaen-17-yl acetate[2]] gave a 14α,17α-oxopropeno intermediate which was readily converted into 14,17α-propanoestra-1,3,5(10)-triene-3,17β-diol **2**, as well as numerous functional-group variants. In order to develop a complementary protocol for the 14β,17β-propano series, cycloaddition of potential 'propyne equivalents' to 3-methoxyestra-1,3,5(10),14,16-pentaen-17-yl acetate was investigated. Methyl propiolate served this purpose, but the requisite multi-step transformation of the resultant cycloadduct, prior to β-bridge oxidative cleavage, was disadvantageous. However, regioselective side-chain functionalisation of 14-allyl-3-methoxyestra-1,3,5(10)-trien-17-one [from acrolein-mediated cycloaddition-fragmentation on 3-methoxyestra-1,3,5(10),14,16-pentaen-17-yl acetate[3]] furnished precursors for intramolecular aldol condensation or reductive cyclisation, leading to 14,17β-propano-14β-estra-1,3,5(10)-triene-3,17α-diol **3**. The results of biological evaluation and structure-activity relationships of these and related hormone analogues are described.

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SYNTHESIS OF STEROID HETEROCYCLES DERIVED FROM CHOLIC ACIDS

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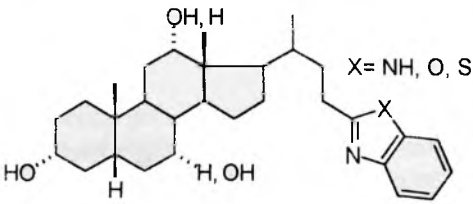
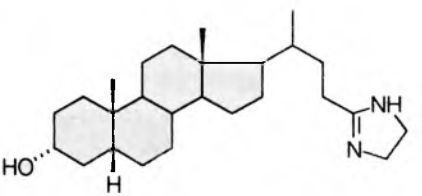
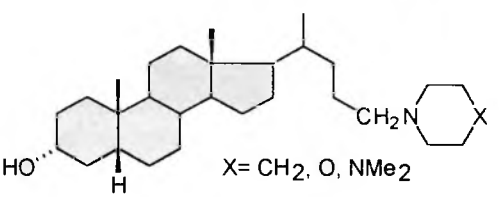
The joint research of our laboratories yielded in several lead structures of interesting biological activity.[1-4] For the recent studies we utilized as starting steroidal material readily available cholic acids (lithocholic, chenodeoxycholic, and cholic) where the natural lipophilic character of the steroid moiety is lowered by the skeletal substitution.



Using Landenburg reaction we synthesized series of benzimidazoles, benzothiazoles and benzoxazoles. We saw interesting biological activity with some leads on L1210, L929, HeLa S3 in 10μmol.l^{-1} giving up to 37% of cell line growth inhibition.



Recently, we were able to accomplish the transformation of cholic acid nitrile to imidazoline just with the direct reaction of nitrile with diamine salt.



Three analogous six membered heterocycles were synthesized alternatively from the lithocholic acid chloride using direct condensation with piperidine, morpholine, and piperazine and subsequent treatment with $LiAlH_4$. The six membered heterocycles were isolated in the form of chloride or methanesulfonyl salt.

All the compounds are now under biological screening. The lead structures will be used for further development of this study.

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BIOSYNTHESIS AND METABOLISM OF BRASSINOSTEROIDS

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Brassinosteroids (BRs) constitute a group of plant steroids which exhibit a variety of plant growth-regulatory activity. Since brassinolide was isolated from rape pollen as the first BR in 1979, more than 30 natural BRs including conjugates have been isolated from a variety of plant species. In general, levels of BRs are high in pollen and immature seed, and, although at lower levels, BRs are present also in vegetative tissues. Naturally-occurring BRs have the following structural features. 1) The side chain carries either a methyl (α or β), methylene, α-ethyl, ethylidene, methylene, or no substituent at C24, and vicinal hydroxyls at C22 and C23. 2) In the B-ring, either 6-oxo, 6-oxo-7-oxa or no functionality is present. 3) In the A-ring, there exist a C3(α or β)-hydroxyl or C2 (α or β), C3 (α or β)-dihydroxy group. These structural features and very recent feeding experiments suggested the following biosynthesis and metabolism of BRs.

1) Major BRs which have a C24α-methyl (brassinolide, castasterone and others) seem to be synthesized from rather minor plant sterols such as campesterol. Sitosterol which has an ethyl group at C24α is generally a most abundant sterol, however, the corresponding BRs are rare. In contrast, BRs which have a methyl group at C24α were most frequently found in higher plants including dicots, monocots, gymnosperms and pteridophytes.

2) Introduction of hydroxyls in the side chain may occur at an early stage of the biosynthesis and precedes modification of A and B rings, because natural BRs have unexceptionally 22*R*,23*R*-vicinal hydroxyls even though functionalities in A and B rings are varied.

3) Brassinolide is synthesized through a sequential pathway of teasterone -> 3-dehydroteasterone -> typhasterol -> castasterone -> brassinolide (Fig. 1)[1,2]. This pathway was established by using crown gall and normal cells of *Catharanthus roseus*. However, it was found to be operating also in seedlings of some higher plants[3].

4) Mutants which have any lesion in biosynthetic pathway are very useful to uncover physiological functions of BRs. To this end, dwarf pea response mutants *lka* and *lkb* whose dwarfism remained to be clarified were analyzed for brassinolide and its precursors, suggesting the possibility that *lka* and *lkb* may be BR biosynthesis

mutants

5) Two types of conjugates, glucosides and fatty acid esters are known. 23-*O*-glucosides are naturally-occurring and a feeding experiment using mung bean cuttings indicated that brassinolide suffers 23-*O*-glucosilation[4]. Recently Abe et al[5] reported the natural occurrence of teasterone-3-*O*-myristate.

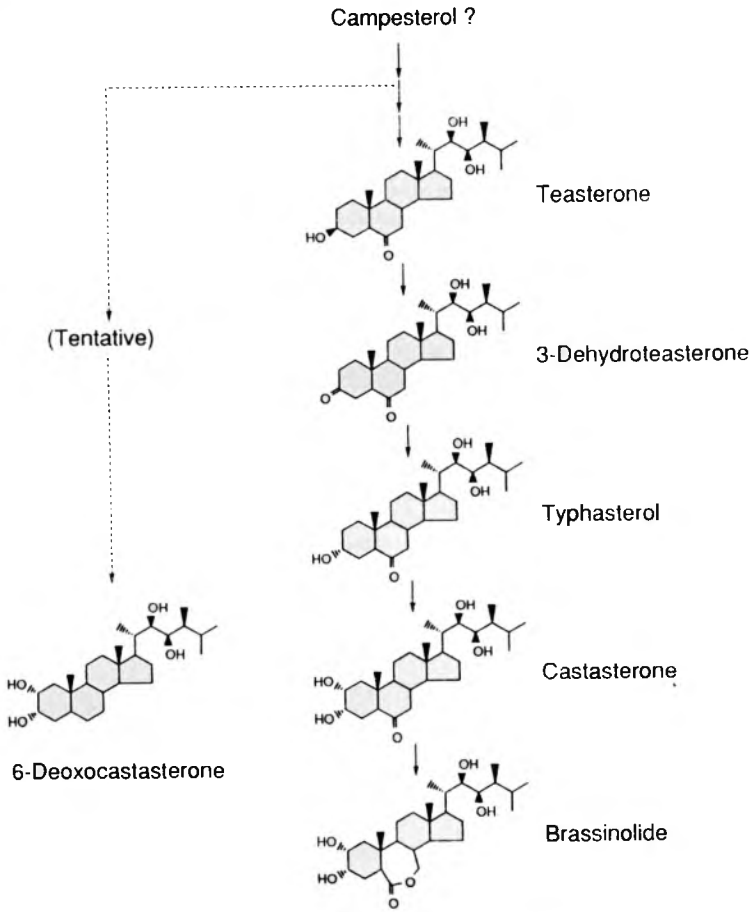

Fig. Biosynthesis of brassinolide

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NEW DEVELOPMENTS IN BRASSINOSTEROID RESEARCH

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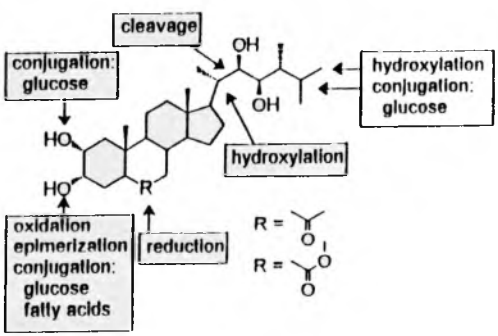
Brassinosteroids are a new class of phytohormones, showing high growth promoting activity as well as other multiple effects on the growth and development of plants. Whereas recently increasing attention has been directed towards the biosynthesis, our interest was focussed more to interconversion and metabolic reactions of this group of steroidal plant growth regulators.

Thus, ^{3}H labelled 24-*epi*-brassinolide and 24-*epi*-castasterone, synthesized from ergosterol, were used to investigate the hitherto unknown pathways of brassinosteroid metabolism in cell suspension cultures of *Lycopersicon esculentum* and *Ornithopus sativus*. Altogether, 26 new brassinosteroid metabolites were isolated from the cultured cells and the medium, and their structures were unambiguously elucidated by detailed NMR and MS investigations. The bioactivity of the key compounds as well as the found metabolic pathways (see, scheme) are also discussed.



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RECENT ADVANCES IN BRASSINOSTEROID RESEARCH

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The investigation of brassinosteroids (BS) during last 10-15 years led to accumulation of great amount of data on this new group of natural phytohormonal steroids[1]. As a result of realisation of large-scale scientific programs started initially in USA and Japan, and later in USSR, Czech Republic, Germany, and China, a number of problems concerned with BS were solved already in the second half of 80's. Among them: i) study of BS spreading in plants, their extraction, structural elucidation, and quantity estimation; ii) discovery of large amount of new representatives of this series; iii) elaboration of synthetic approaches which, generally, provide accessibility of BS for biological study. Especially important, in contradistinction with early forecast[2], the finding of new active BS and advances in chemical synthesis led to creation of economically covered approaches to practical BS application in agriculture to increase plant productivity[3,4]. Obtained data present a reliable basis for further development of BS investigation in theoretical and practical respects. These also considerably enrich the ideas on the role of steroids as universal carriers of biological information for all leaving organisms.

The present stage in BS investigation is characterised by deep attention to further improvement of preparative technologies, first of all, for those BS which can be considered as most perspective for practical application: 24-epibrassinolide, 28-homobrassinolide, brassinolide. In the last case all known methods of synthesis are not efficient enough and have only scientific interest, that is why the elaboration of practically valuable synthetic approaches is still actual. Another important aspect of synthetic investigations is the preparation of new BS analogues with better biological properties. Their employment could become the strategic direction in BS usage as in the case of most applications of steroids in medicine. The strengthening of applied orientation of the work in BS area is concerned with i) the essential widening of physiological, biochemical and other works directed on the creation of optimal technologies of BS application in agriculture; ii) the broadening of range of studied agricultural plants; iii) the clarifying of the conditions in which extreme activity of BS can be achieved; and iv) the discovery of new effects of BS.

In the lecture recent advances in chemical synthesis of BS will be discussed. In particular, special attention will be paid to some new approaches to brassinolide synthesis *via* heterocyclic intermediates, unsaturated sulfones, and some other key compounds, together with advanced synthesis of 24-epibrassinolide including the approach based on using of chiral catalysts for hydroxylation of the side chain. The results of BS practical application in a agriculture will be briefly summarised.

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STRUCTURAL REQUIRIMENTS FOR HIGH BRASSINOSTEROID ACTIVITY: A GEOMETRICAL APPROACH.

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In the field of brassinosteroids, potent plant growth regulators, different qualitative structure-activity relationships have been established taking into account the activity data obtained in special bioassay systems[1,2,3]. Nevertheless, from our point of view, the requirements postulated for a high brassinosteroid activity are so far to become general limiting the scope of applicability to the functionalities presents in the group of brassinosteroids tested. As an example, there was no prove to discard brassinosteroids with A/B *cis* ring junction and/or 2β,3β diol.

In this sense, we have synthesized new analogs with these kind of functionalities eliciting some of them high activity in rice lamina inclination bioassay which are in some case similar to that showed by 28-homobrassinolide[4,5,6]. Also we have found that the activity of brassinosteroids with 22S,23S diol strongly depends on the alkyl substituent at C_{24}[6]. Both findings indicate the weakness of the requirements postulated.

Assuming that brassinosteroids may act at molecular level through a mechanism similar to that of animal steroid hormones, a receptor/ligand complex which binds to nuclear or cytoplasmatic sites to regulate the expression of specific genes should be involved. Therefore, the active brassinosteroids should have a single three-dimensional conformation that we call *"active conformation"* which fits the receptor/s. It is in this sense that we focus the way to define the structural requirements for a high brassinosteroid activity.

In order to find this conformation, a systematic conformational analysis employing molecular mechanics approximations has been developed over 23 compounds with different functionalities in A/B ring and side chain. As a result of this study, different flexibility for each type of side chain has been observed.

In this communication we will present the methodology used to get the *"active conformation"* for each compound and how it can be related to the activity. This method is based on distances between different functional groups, RMS index and molecular shape analysis. Taking into account the results obtained, a more rigorous way to establish the structural requirements for a high brassinosteroid activity will be presented.

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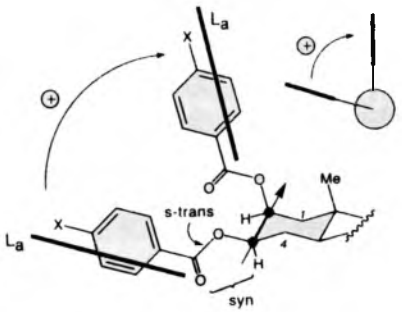
EXCITON COUPLED CIRCULAR DICHROISM WITH ENHANCED SENSITIVITY FOR MICROSCALE STRUCTURAL STUDIES

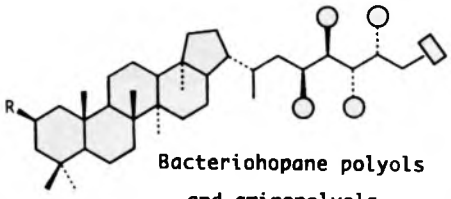
Ning Zhao, Stefan Matile, Verena Dirsch, Koji Nakanishi, and Nina Berova

Department of Chemistry, Columbia University, New York, N.Y. 10027



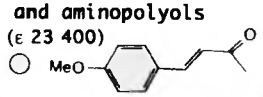
The dibenzoate chirality rule for 1,2-glycols, introduced by Harada and Nakanishi in 1969, has continued to make the progress over the years and now as Exciton Coupled Circular Dichroism (ECCD) is one of the most powerful method for the study of molecular chirality in solution. Some examples will be presented from recent studies which have focused in extending ECCD to unexplored areas by developing new strategies and chromophores for structure elucidation on submicrogram scale.

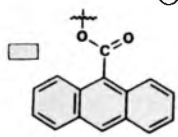
I. Exciton coupling between different chromophores (selective derivatization to discriminate O,N-functions) and CD measurements, both in polar / nonpolar solvents, is a versatile approach for structural analysis of acyclic, conformational flexible molecules:



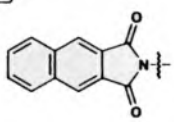
Bacteriohopane polyols and aminopolyols

307 nm (ε 23 400)



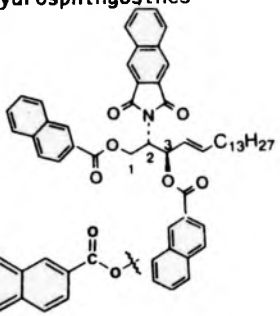


λex = 252 nm (ε 140 000)
λem = 456 nm



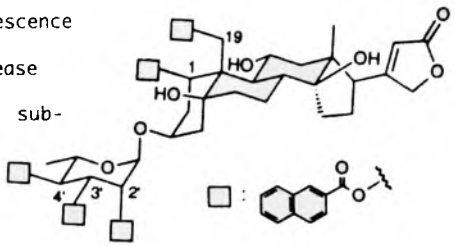
λex = 258 nm (ε 64 500)
λem = 367 nm

Sphingosines and Dihydrosphingosines



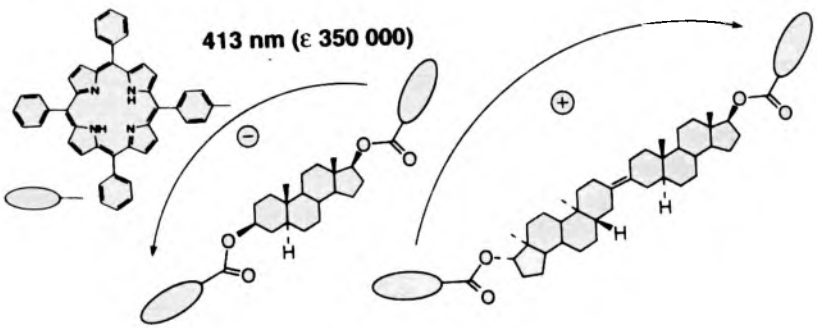
λex = 234 nm (ε 43 000)
λem = 374 nm

II. CD chromophores with fluorescence and intense UV absorption increase sensitivity and reinforce the sub-microscale structural analysis



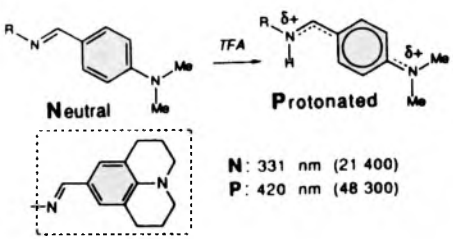
Cardenolides: Ouabain

III. Tetraarylporphyrins, with extremely untense UV-VIS absorptions, enhance the CD sensitivity and are powerful chromophores for long range exciton coupling (up to 50-55 Å)

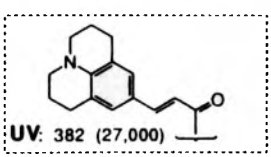


IV. New "red-shifted" CD chomophores give "clean" UV-VIS exciton couplets outside the substrate attributed spectral region

Chromophores for primary amino groups



Chromophores for hydroxyl groups



FURTHER INVESTIGATIONS ON BIOMIMETIC REARRANGEMENTS OF LANOSTEROL DERIVATIVES

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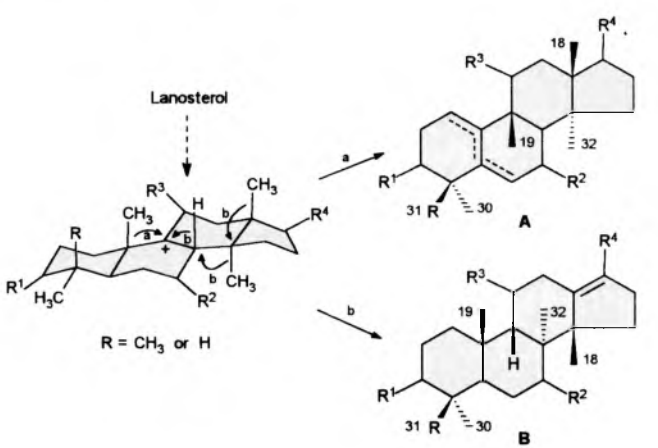
Skeletal rearrangements of steroids and triterpenes have been intensively investigated. Hovewer, little was known about reactions proceeding with formation of carbocationic centre at carbon C(9) of steroids. Generation of the carbocation in position 9 of lanostane in the acid-catalysed reaction of an alcohol or epoxide might induce biomimetic rearrangement leading to related triterpenes with carbon skeleton of cucurbitane **A** and/or protostane **B** (Scheme, path **a** or **b**). Compounds belonging to these groups are of synthetic and biological importance. Mechanistic and stereochemical aspects of rearrangements are also of interest. Several attempts to synthesize substrates suitable for rearrangements failed or gave poor results.

An effective 10-methyl group migration was achieved in the BF_3-catalysed reaction of 9β,11-epoxy-7-oxo-5α-lanostane derivatives. The electron-withdrawing carbonyl group in position 7 supressed migration toward ring D. By this approach several 19(10→9β)*abeo* steroids were prepared including true cucurbitacin functionalized in the side chain. It was also shown, that configuration of C(9) in 9,11-epoxides is important for the direction of rearrangements. The other factor, steric compression between axial methyl groups at C(4) and C(10), was found to be not essential for 10-methyl group migration. Thus, 4β-demethyl-7-oxo-9β,11-epoxy-5α-lanostanes rearranged to 31-norcucurbitanes, while position of the double bond in products was dependent on the functional group at C(3). For example, the 3β-acetate gave the 11β-acetoxy-31-nor-cucurbita-3,5-diene-7-one as the main product, while the 3-ketone rearranged to the 3,7-diketone with the unconjugated double bond in position 1(10). The rearrangement of 4β-demethyl-9α,11-epoxy-5α-lanostan-3-one resulted in formation of fusidenone (31-norprotostane), a convenient substrate for the synthesis of fusidic acid, a steroid antibiotic. Finally, rearrangements of 9β,11-epoxy-5α-lanostanes with 7α- or 7β-acetoxy group gave products suggesting participation of the 7α-substituent in stabilization of the transient carbocation(s).

In conclusion, the rearrangements are susceptible to changes in substituents and/or stereochemistry at C(3), C(7), C(9) and C(11). The appropriate functionalization of the substrate 9,11-epoxide enables preparation of cucurbitane and protostane derivatives, including 31-nor analogs, in high yield.



SEEKING FOR ANTIFEEDANTS OF NATURAL ORIGIN

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The chemical defence mechanism of mushrooms of *Lactarius* family involves the enzymatic transformation of velutinal (precursor) into a series of sesquiterpenes of marasmane and lactarane skeletons. The transformation is triggered by mutilation of the body of mushroom, where a cascade of reactions is initiated. In our investigation of ethanolic extracts of mushrooms of *Lactarius* family we have isolated and established structures of about 60 compounds, and we found that all of them showed pronounced antifeedant activity against storage pests: *Tribolium confusum*, *Trogoderma granarium* and *Sitophylus granarius*. After having examined the antifeedant activity of a such number of compounds conclusions on structure activity relationship were drown.. It was found that an artifact 3-O-ethyl-furandiol possessed a very strong activity[1]. The seven and five membered ring junction in all natural lactaranes is cis, however it is possible to change the ring junction into trans and to prepare trans fused derivatives[2]. The synthesis of trans fused 3-O-ethyl-lactaranes and their antifeedant activity against the storage pests will be presented.

The second part of the lecture involves isolation and structure elucidation of limonoids from plants of *Meliaceae* family. Structures of some new compounds from *Entandrophragma utile*, *E. candolei* and *E. cylindricum*, and their antifeedant activity will be discussed.

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STUDIES OF BIOACTIVE "TERTIARY" MUSHROOM METABOLITES

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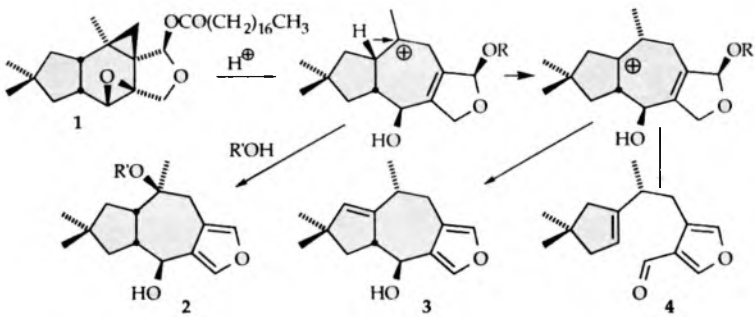
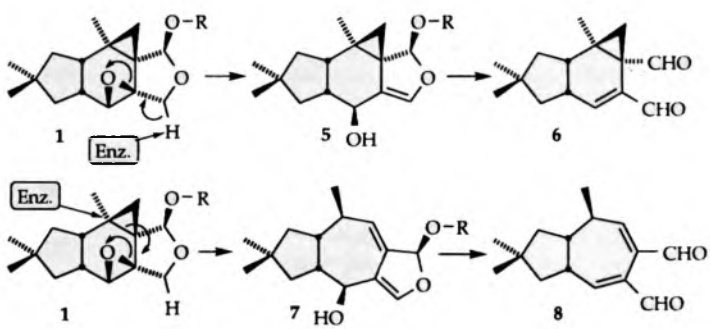
In the fruit bodies of the pungent *Lactarius* species (milk caps), reactive and biologically active sesquiterpenes are formed enzymatically in seconds as a response to injury. The precursor is the marasmane velutinal, esterfied with a fatty acid (e.g. stearoylvelutinal **1**) and present in the latex of the fruit body as an emulsion. The velutinal esters are perfectly stable in the intact mushroom, but the isolated velutinal esters or free velutinal are very sensitive to traces of acid and their half-lifes in reagent grade protic solvents (e.g. methanol) are only a few minutes. Careless extraction of *Lactarius* fruit bodies has certainly led to the isolation of artifacts (e.g. the furans **2**, **3** and **4**) formed from the acid catalysed degradation of the velutinal esters.



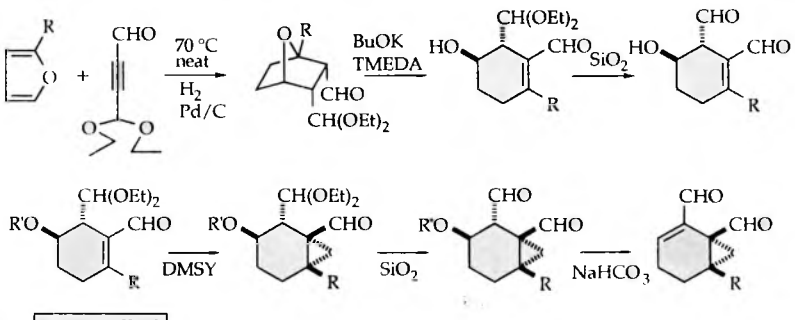
The enzymatic conversions of the velutinal esters yields unsaturated dialdehydes, possessing potent antibiotic and antifeedant activities and believed to be the products of a natural binary chemical defence system. The biosynthetic conversions have been studied, and the two unsaturated dialdehydes isovelleral (**6**) and velleral (**8**) are probably formed via the intermediates **5** and **7**.

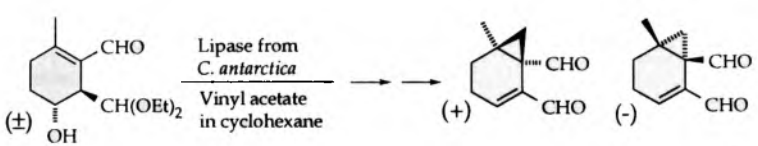


Terpenoids containing an unsaturated 1.4-dialdehyde group have also been isolated from completely different organisms, like plants, insects and molluscs, and

it appears as if evolution has developed this useful functionality in several independent branches. The chemical reactivity of the unsaturated dialdehyde functionality has been suggested to be responsible for the biological activities of these compounds. However, several different reactions with the unsaturated dialdehydes have been observed, e.g. nucleophilic addition to the α,β-unsaturated aldehyde and the formation of pyrrole derivatives via the reaction of both aldehyde groups with a primary amino function, and some of the compounds are rapidly autoxidised to reactive products. As a group of bioactive natural products, the unsaturated dialdehydes are therefore not as homogenous as one could believe, which has hampered the QSAR studies performed.

Several of the natural unsaturated dialdehydes have been synthesised, some even in a enantiomeric pure form. Also simpler analogues containing the unsaturated dialdehyde group have been prepared, in order to facilitate QSAR studies.





Contrary to the various stereoisomers of isovelleral (**6**), the two synthetic enantiomers lacking the cyclopentane ring are exactly equally active, and the indication that the absolute stereochemistry of the dialdehyde functionality of isovelleral (**6**) is important for its bioactivity has so far not been confirmed. Interestingly, the synthetic analogue of isovelleral (**6**) lacking both the cyclopentane ring and the methyl group next to the cyclopropane ring is much more potent.

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RECENT ADVANCES IN THE MEDICINAL CHEMISTRY OF TAXOID ANTITUMOR AGENTS

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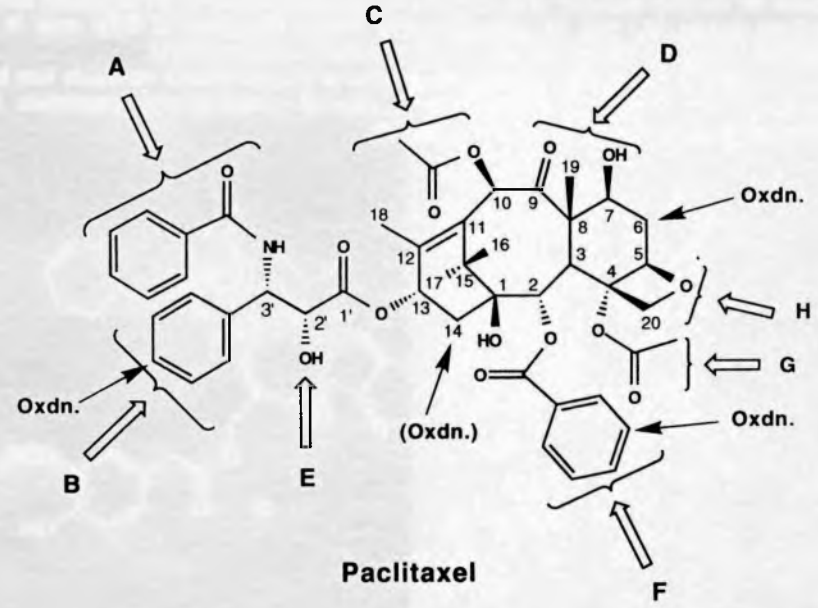
New York 11794-3400, U. S. A.

Taxol® (paclitaxel) is currently considered the most exciting lead in cancer chemotherapy. Taxotere® (docetaxel), a semisynthetic analog, is also exceptionally promising. Paclitaxel and docetaxel possess strong antitumor activity against different cancers which have not been effectively treated by existing antitumor drugs. Paclitaxel has been approved by FDA for the treatment of advanced ovarian cancer (1992) and breast cancer (1994), and is currently in phase II clinical trials for lung, neck and other cancers. Docetaxel is currently in phase II and III clinical trials for breast, lung and other cancers. Recent reports on clinical trials of paclitaxel and docetaxel, however, have disclosed that these highly effective drugs have a number of undesired side effects as well as multi-drug resistance (MDR). Therefore, it is very important to develop new taxoid anticancer drugs which have less undesirable side effects and MDR, better pharmacological properties and/or tumor specificity different from those of the parent drugs. This lecture will describe our rational approaches to the development of such new anticancer drugs based on the structure-activity relationship study of taxoids as well as NMR study on the hypotherical bioactive conformation.



DESIGN, SYNTHESIS AND ACTIVITY OF PHORBOL-BASED LIGANDS FOR THE VANILLOID RECEPTOR

Giovanni APPENDINO,[a] Giancarlo CRAVOTTO,[a] Giovanni PALMISANO,[a] Arpad SZALLASI[b]

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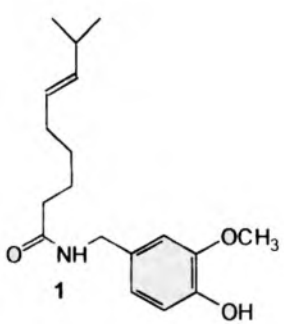
Capsaicin (**1**), the pungent principle of hot pepper, excites and then desensitizes a subset of sensory neurons involved in nociception and neurogenic inflammation. Desensitization to capsaicin is thought to have a therapeutic potential, but the use of capsaicin is severely limited by its irritancy. The synthesis of capsaicin-like compounds with an improved desensitization/irritation ratio has been pursued for decades. These efforts have been further stimulated by the recent discovery of a naturally occurring ultrapotent agonist [resiniferatoxin (RTX, **2**)] and by the synthesis of a competitive antagonist (capsazepine). RTX and capsaicin are structurally and biogenetically unrelated, but share a homovanillyl moiety essential for their biological activity. The common membrane recognition site for these compounds was thus named the "vanilloid" receptor.

The structure-activity relations of capsaicinoids have been explored in depth, but the central elements of the RTX pharmacophore are still ill defined. We have investigated the possibility of obtaining vanilloids structurally related to RTX using phorbol as starting material. This compound can be obtained in an averaged yield of 1% from croton oil, and its diterpene core differs from that of RTX only in the oxygenation pattern of ring C and for the presence of an intact ring D.

An efficient metodology for the preparation of phorbol-based vanilloids has been developed, and some of these compounds showed unique binding properties. New rearrangements of phorbol were also observed.



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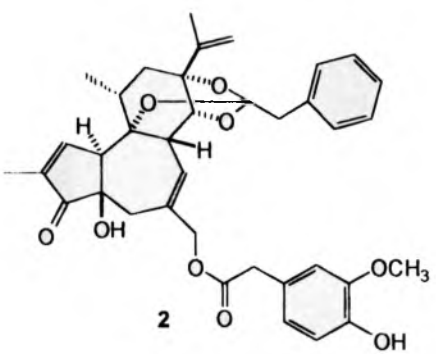

2

CHEMISTRY OF ANT PHEROMONES

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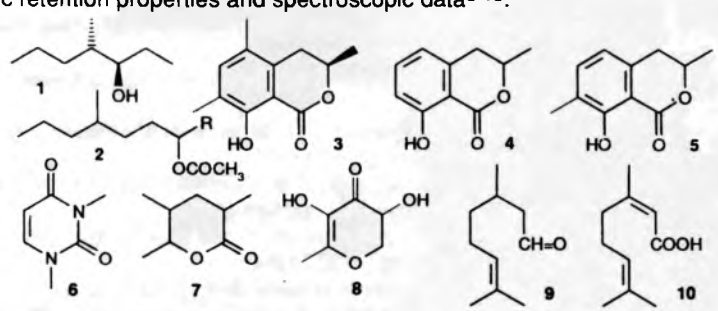
A *pheromone* is a semiochemical, usually a glandular secretion, used within a species; one individual releases the chemical as a signal and the other responds after smelling or tasting[1]. Ants (Hymenoptera: Formicidae) employ the most complex forms of chemical communication of any animals, their complex social behavior appears to be mediated in large parts by pheromones and chemoreception[2].

The typical ant worker is a walking battery of exocrine glands, more than ten of such organs have been implicated in the production and release of semiochemicals. Mixtures of terpenoids are found, as well as straight-chain hydrocarbons, functionalized compounds like alcohols, aldehydes and ketones, aromatic and heterocyclic substances, etc., and divergently used as trail and recruitment pheromones, alarm substances, sex pheromones, nest and territorial marking substances and defense secretions.

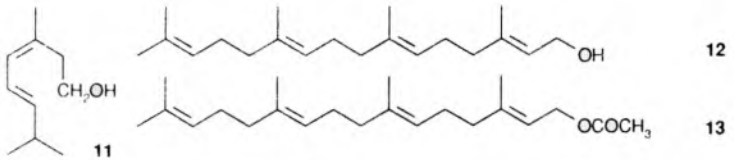
By means of a gc solid-sample injector[3] a single insect's glands or body sections can be gaschromatographically investigated for volatile constituents. Preferentially, the electroantennogramm EAG, adapted to a gas chromatograph, is used as a biological gc detector[4]. With these two powerful analytical tools, nanogram and even subnanogram amounts of biologically active substances can be analyzed and their chemical structures elucidated.

Focussing on trail and recruitment pheromones of the ant subfamilies Formicinae, Myrmicinae and Ponerinae, pheromonal constituents of various species of the genus *Lasius, Formica, Camponotus* and *Megaponera* (Formicinae), *Manica* and *Pristomyrmex* (Myrmicinae), *Leptogenys, Pachycondula* and *Ectatomma* (Ponerinae) were identified (Scheme 1 and 2) and characterized by chromatographic retention properties and spectroscopic data[5-15].



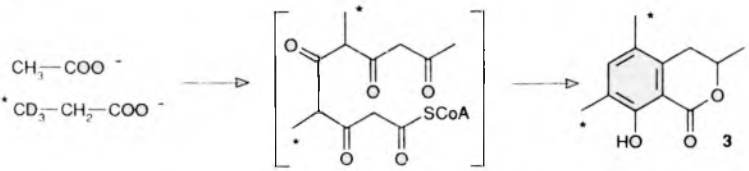
Scheme 1: Trail pheromone constituents of **1** *Leptogenys diminuta* and *L. species 5*, **2** *Leptogenys peuqueti*, **3** *Lasius niger* and *Camponotus silvicola*, **4** *Formica rufa*, **5** *Camponotus rufipes*, **6** *Megaponera foetens*, **7** *Camponotus herculeanus*, **8** *Lasius fuliginosus*, **9** *Pachycondula commutata*, **10** *Camponotus floridanus*.

All the compounds were synthesized and the synthetic chemicals electrophysiologically tested for biological activity with electroantennogram and single cell recordings as well as in laboratory behavioral bioassays.



Scheme 2: Recruitment pheromone components of **11** *Leptogenis diminuta*, *L. species 5* and *L. peuqueti*, **12** and **13** *Ectatomma ruidum*

Selected feeding experiments with deuterium marked acetate and propionate as well as specifically labelled mellein **4** revealed an acetate-propionate biogenetic pathway for the corresponding dihydroisocoumarin (e.g. Scheme 3)[16] and pyranone pheromones.



Scheme 3: Biogenetic pathway to dihydroisocoumarin pheromone **3**.

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SYNTHETIC STUDIES ON SAPONACEOLIDES AND THEIR ANTITUMOR ACTIVITY

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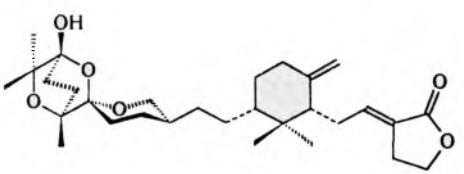
Saponaceolides A-D, isolated[1,2] from the fruiting-bodies of *Tricholoma saponaceum* (Basidiomycetes) possess an unprecedented skeleton and an interesting antitumor activity *in vitro* on several tumor cell lines. Saponaceolides contain an α,β-unsaturated lactone moiety which is often responsible for the biological activity of many natural compounds.
During our synthetic approach to saponaceolide B (**1**) we have completed the enantioselective synthesis of model compound **2**, which corresponds to the right half of the entire structure of **1**. Key steps of the synthesis of **2** include i) the stereoselective preparation of a trisubstituted olefin; ii) the Sharpless asymmetric dihydroxylation of a homomonoterpene ester, and iii) the biomimetic cyclisation of an epoxyallylsilane. An advanced intermediate in this synthesis can be an useful chiron for the preparation of several monocyclofarnesane sesquiterpenes.



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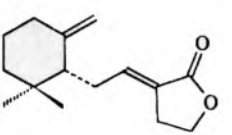

2

The simple lactone **2** was much less active than saponaceolide B (**1**) in simple biological tests, indicating that the entire structure is necessary for maintaining the biological activity.

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A PHYTOCENTRIC OVERVIEW OF THE ECOLOGICAL ROLES OF HIGHER PLANT TERPENOIDS

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Characteristics of higher plant terpenoids that result in mediation of numerous kinds of ecological interactions will be discussed. A terrestrial phytocentric approach will be necessary because of the magnitude and scope of terpenoid interactions. The importance of the occurrence of terpenoids as mixtures, either constitutive or induced, their qualitative and quantitative compositional variation and dosage dependent effects will be emphasized. Selected phytocentric examples will be presented of terpenoid interactions: 1) defense against generalist and specialist insect and mammalian herbivores, 2) defense against insect-vectored fungi and potentially pathogenic endophytic fungi, 3) attraction of entomophages and pollinators, 4) allelopathic effects that inhibit seed germination and soil bacteria, and 5) interaction with reactive troposphere gases. The results of these interactions will be integrated by discussing how the terpenoids are contributing factors in determining some properties of terrestrial plant communities and ecosystems.

ECDYSTEROIDS FROM DIFFERENT VARIETIES OF *AJUGA REPTANS*

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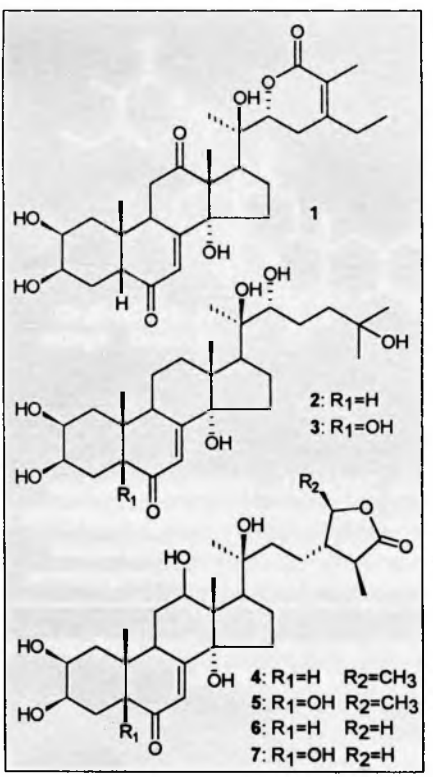
Seven ecdysteroids have been previously reported as major components of *Ajuga reptans*[1]: ajugalactone (**1**), cyasterone (**4**) and sengosterone (**5**) with a C29 skeleton, 29-*nor*-cyasterone (**6**) and 29-*nor*-sengosterone (**7**) with a C28 skeleton and 20-hydroxyecdysone (**2**) and polypodine B (**3**) with a C27 skeleton. The total content and relative composition of these phytoecdysteroids were very diverse in different parts of normally grown or *in vitro* micropropagated plants.[2,3]

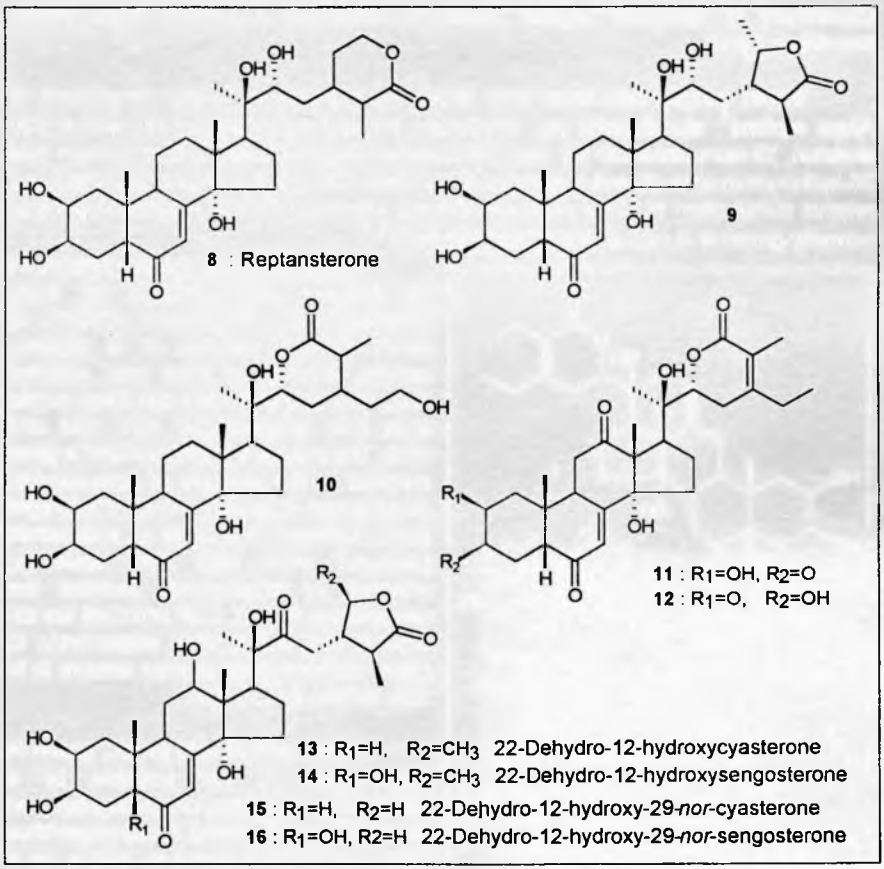


As an extension of these studies, in the present communication we have investigated the ecdysteroid contents of seven different varieties of *A. reptans*, namely, from green ones (var. *reptans*: AR2, AR3, AR5 and AR7) and three purple ones (var. *atropurpurea*: AR1, AR4 and AR6). The results obtained showed a great variation in the amounts of ecdysteroids, ranging from 1239 ppm (AR3) to 2629 ppm (AR5). Likewise, the types of ecdysteroid produced were also very dissimilar, whereas in atropurpurea varieties the percentages of C-27, C-28 and C-29 are 33-51%, 17-40% and 27-32%, respectively, in green varieties these profiles amount to 0-10%, 44-69% and 23-56%.

From roots of AR5 plants, grown in greenhouses, it was possible by a combination of column chromatography on silica gel and repeated micro-preparative HPLC on reversed phase to isolate, as a minor component, a new ecdysteroid that was characterized by homonuclear and heteronuclear bidimensional NMR spectra (COSY and HETCOR) and comparison with spectral data (NMR, IR, MS, UV) of authentic samples of structurally related ecdysteroids. The new compound named reptansterone (**8**), displayed an unprecedented structure with a δ-lactone in the side chain.

Likewise, from the roots of atropurpurea variety AR6, grown in the field and collected in autumn[4], by application of the same chromatographic and spectral techniques, other new minor components were identified as 28-*epi*-sengosterone (**9**),

5,29-dihydroxicapitasterone (**10**), 3- and 2-dehydroajugalactone (**11,12**). Finally, from the aerial parts of the same variety we isolated a new series of four 12-hydroxy-22-dehydroecdysteroids (**13-16**), structurally related to cyasterone and sengosterone and their cooresponding nor-derivatives, respectively.



Some relevant spectral data, possible ecological and biosynthetic implications of the occurrence of all these compounds in the plant will be discussed.

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VOLATILE HOMOTERPENES IN THE PLANT'S DEFENSE; BIOSYNTHESIS AND INDUCTION OF A UBIQUITOUS SYNOMONE

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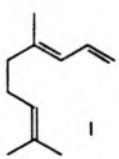
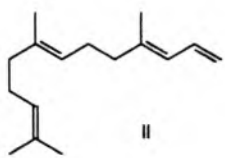
The two homoterpenes 4,8-dimethylnona-1,3,7-triene **I** and 4,8,12-trimethyltrideca-1,3-7,11-tetraene **II** are structurally simple volatiles endowed with interesting biological functions. They are released from many night-scented flowers and a possible role in the attraction of pollinators has been discussed. On the other hand **I**, **II** or both compounds may be emitted from leaves of many angiosperms if they are damaged by a herbivore. In at least two well documented cases[1] they are involved in tritrophic interactions as signal compounds guiding carnivorous mites or wasps to their prey, like for example foraging spider mites or beet army worms.



I

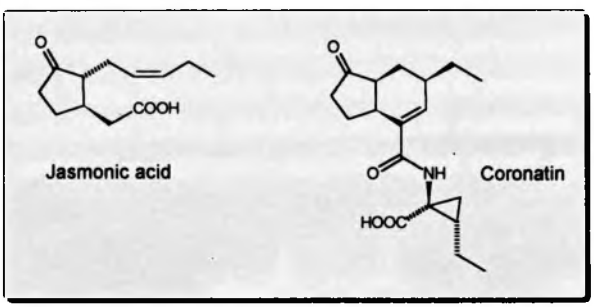


II

The precursors of **I** and **II** are the regular sesquiterpenoid nerolidol and the diterpene geranyllinalool. Feeding studies with deuterium labelled precursors and leaves of the Lima bean (*Phaseolus lunatus*) suggest that (3*S*)-nerolidol is degraded to **I** in a stepwise fashion[2]. Following oxidation to 1,2-epoxinerolidol and hydrolysis to 1,2*R*,3*S*-trihydroxy-3,7,11-trimethyldodeca-6,10-diene geranylacetone originates from oxidative cleavage of the trihydroxy compound. A final deacylation of the ketone generates the homoterpene **I** and acetic acid as the formal second product. The transformation appears to be achieved by **P**-450 type enzymes and resembles the dealkylations of the steroid metabolism[3].

Feeding herbivores induce the biosynthesis of **I**, **II** or other volatiles by introducing bioactive components with their saliva into the damaged leaves. Carbohydrate cleaving enzymes like e.g. ß-glucosidase (from bitter almond), pectinases and cellulases are especially effective and generate in Lima beans virtually the

same pattern of volatiles than the feeding spider mites (*Tetranychus urticae*)[4]. Besides of the carbohydrate cleaving enzymes several compounds of the octadecanoid signalling pathway are able to induce the biosynthesis of volatiles. The most active compound is jasmonic acid (ca. 50 nmol ml^{-1}) which triggers the production of volatiles in many plants ranging from archetype (ferns) to highly evolved plants (angiosperms)[5]. Early intermediates of the biosynthetic pathway leading to jasmonic acid (e.g. 12-oxo-PDA) are also active, but trigger only a selected pattern of compounds.



Besides of **Ja** the phytotoxin coronatin is another very active elicitor of odor production (ca. 1 nmol ml^{-1}). Induction experiments with synthetic conjugates of **JA**- and coronatin-analogues with amino acids (e.g. Ile, Leu) suggest that also the amino acid conjugates may have an important role in the regulation of secondary pathways in plants.

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Characterization of 2-methyl-1,3-butadienyl and (1-methylene)-3-propenyl moieties in volatile compounds by Gas chromatography-Infrared Spectrometry: Application to Farnesene Isomers

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The structural moieties CH_2=CH-CH(CH_3)=CH-CH_2- and CH_2=CH-C(=CH_2)-CH_2- are frequently encountered in terpenes. Commonly, terpenes bearing these skeletal fragments are referred to as α– and β–type compounds. Vapor-phase infrared spectra allow the recognition of these two moieties. For α-type groups, even the cis/trans configuration of the trisubstituted double bond can be determined unambiguously. An absorption at 3094-3091 cm^{-1}, for the =CH_2 stretch vibration, is common for both these groups, however, due to the presence of two =CH_2 groups, the relative intensity of the band is much higher for β-type compounds. For α-type compounds, a cis configuration at C-3 carbon atom is characterized by a =CH_2 wag absorption at 907-906 cm^{-1}. For β-type compounds and (3*E*)-α-type compounds, this band appears at 899-898 cm^{-1}. In addition, a wavy "fingerprint" pattern with three minima at 1660, 1632, and 1595-1594 cm^{-1}, the intensities of which gradually increase as frequencies decrease, is characteristic for β-type compounds. Our generalizations are based on spectra of cis and trans ocimene, myrcene, and dehydration products of many 3-methyl-1-alkene-3-ols. The deductions made were used to characterize the six isomers of farnesene by GC-FT/IR. Furthermore, this method allows the characterization of 3*E* and 3*Z* bonds in other farnesene congeners. For example, homo- and bishomofarnesene isomers from Myrmica ants were shown to bear a 3*Z* bond.

Novel aspects of the microbial hydroxylation of alicyclic compounds - a tool for the synthetic chemist.

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Abstract not received

ACETOACETYL-COA THIOLASE AND HMG-COA SYNTHASE ACTIVITIES IN THE BIOSYNTHESIS OF MEVALONATE IN *CATHARANTHUS ROSEUS*

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Plant terpenoids are a diverse group of primary and secondary metabolites. Most of these compounds have an important physiological function or play a role in the interactions of the plant with its environment. In the biosynthesis of terpenoids, mevalonate is an important intermediate. At present much research is focused on the regulation of the mevalonate biosynthesis in order to manipulate the accumulation of the mevalonate derived metabolites.

In yeast and animal cells, mevalonate is formed from three molecules of acetyl-CoA, in a series of reactions catalysed by the enzymes acetoacetyl-CoA CoA thiolase (AACT), HMG-CoA synthase (HMGS) and HMG-CoA reductase (HMGR, fig 1). HMGR is well characterized from various sources, including several plant spcies. So far little is known of the plant AACT and HMGS. However, it was reported that in radish and maize seedlings the formation of HMG-CoA from three molecules of acetyl-CoA could be catalysed by one single protein (fig. 1, [1]).

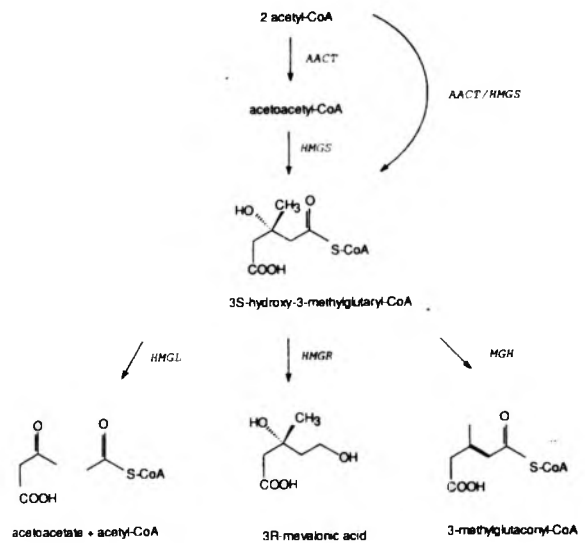

Figure 1. *Enzymes involved the formation and degradation of 3S-hydroxy-3-methyl- glutaryl-coenzyme A (HMG-CoA).* AACT, acetoacetyl-CoA thiolase; HMGS, HMG-CoA synthase; AACT/HMGS, enzyme described by Weber and Bach, 1994; HMGR, HMG-CoA reductase; HMGL, HMG-CoA lyase; MGH, 3-methylglutaconyl-CoA hydratase.

The formation of HMG-CoA was studied in *Catharanthus roseus*, a model organism for studies on the regulation of metabolic pathways, among others the formation of terpenoid indole alkaloids. Some newly developed HPLC methods were used to characterize the enzyme activities involved in the metabolism of HMG-CoA [2]. It was demonstrated that for the biosynthesis of HMG-CoA both AACT and HMGS activity were required. Both enzymes showed similar chromatographic behaviour [3]. AACT has been purified from suspension cultured *C. roseus* cells by affinity and ion-exchange chromatography (purification factor 1400). SDS-PAGE showed a molecular mass of 41 kDa for the subunit. The purest fraction was free from HMGS activity. HMGS was partially purified by affinity chromatography, ion-exchange and size exclusion chromatography. The purest fraction was not free from AACT activity.

Besides for HMGR, HMG-CoA is also a substrate for two other specific enzymes, i.e. HMG-CoA lyase (HMGL) and 3-methylglutaconyl-CoA hydratase (MGH, fig 1). Both were partially purified from *C. roseus* suspension cultured cells. Some further aspects of HMG-CoA metabolism in *C. roseus* will be discussed.

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ENZYMATIC CYCLIZATION OF OXIDOSQUALENE AND SQUALENE

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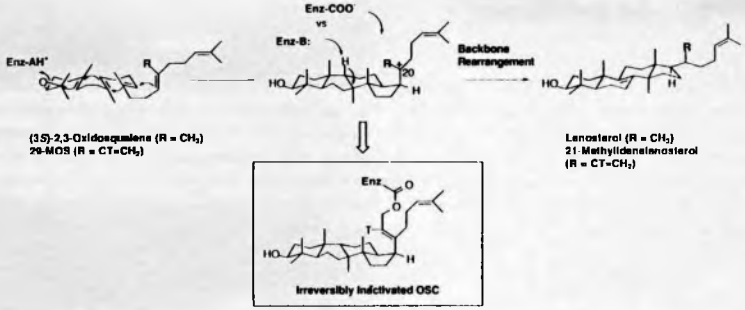
Enzymatic cyclization of oxidosqualene and squalene are the most remarkable steps in the biosynthesis of sterols and triterpenes. The acyclic polyenes are converted to a variety of polycyclic products by different enzyme systems. The mechanistic and evolutionary aspects of the cyclization reactions have intrigued chemists and biochemists for many years.[1] Further, the regulation of cyclase levels *in vivo* has clinical importance for modulation of cholesterol biosynthesis.[2]

Vertebrate Oxidosqualene Cyclase

At Stony Brook, we have been working on vertebrate oxidosqualene cyclases (OSCs) and recently completed molecular cloning and functional expression of a cDNA for rat liver OSC.[3] A 2.2 kbp open reading frame encodes a 83 kDa protein with 733 amino acids. The deduced amino acid sequence of the rat enzyme showed significant homology to those of the known OSCs from yeast and plant (39-44% identity), and still retained 17-26% identity to two bacterial squalene cyclases (SCs), suggesting the ancient lineage of this vertebrate enzyme to ancestral eukaryotic and prokaryotic cyclases. Like other cyclases, the rat enzyme contains highly-conserved regions with a repetitive β strand-turn motif, rich in aromatic amino acids (the 'QW' motif), which likely serves a structural or catalytic functional role in the cyclization reaction.[4] The hydropathy plot analysis revealed rat OSC is a moderately hydrophilic protein, however, this microsomal membrane-associated enzyme showed no clearly delineated transmembrane domain.

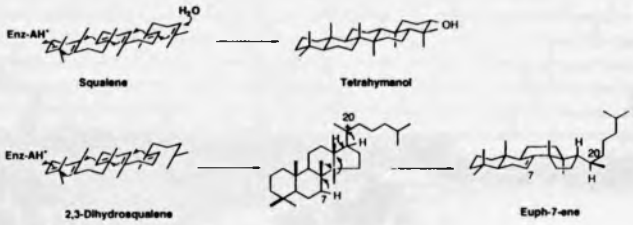


29-Methylidene-oxidosqualene (29-MOS) is a mechanism-based irreversible inhibitor specific for vertebrate OSCs.[5,6] Active-site mapping of rat liver OSC using [^{3}H]29-MOS revealed that the binding site sequence at the N-terminal of the QW-4 motif is highly conserved in all known OSCs, and that Asp^{456} residue was labeled with the suicide substrate.[7,8] The initially formed 21-methylidene-protosterol cation would be trapped by nucleophilic attack by the carboxyl group of the Asp residue, resulting in ester bond formation.

Bacterial Squalene Cyclases

In some bacteria and protozoa, squalene is cyclized directly (*without* prior epoxidation) to pentacyclic triterpenes such as tetrahymanol and hop-22(29)-ene.[1] The cyclization reaction is rather simple, and proceeds in all pre-chair conformation of squalene without carbon skeletal rearrangement. At Mulhouse, we have recently demonstrated that only a small modification of the substrate can cause a dramatic change in the cyclization mechanism of tetrahymanol synthase and hopene synthase,[9,10] which is of great interest from the view point of the molecular evolution of the cyclases.

2,3-Dihydrosqualene (SqH_2) is a molecule lacking one of the terminal double-bond of squalene, therefore making it impossible to form the pentacyclic framework. Tetrahymanol synthase from *Tetrahymena pyriformis* converted SqH_2 to tetracyclic triterpene, euph-7-ene with unexpected skeleton and back-bone rearrangement. On the other hand, hopene synthase from *Alicyclobacillus acidocaldarius* cyclized SqH_2 into a 1:1 mixture of (20*R*)-dammar-13(17)-ene and (20*R*)-dammar-12-ene. In both cases, the cyclization produced only transposed dammarenes with a five-membered D-ring, and all cyclization products had only the 20*R* configuration suggesting the stereochemistry of the cyclization reactions must be strictly controlled by the enzymes. Formation of a five-membered D-ring instead of a six-membered D-ring is apparently only induced by the lack of participation of the π-system from the terminal double-bond.



Squalene is converted to various skeletal types of triterpenes by different enzymes which employ only small variations of a single catalytic mechanism. Such variations have been considered to be attained by only small modification of the structure of the active site of the enzyme. The versatility of the above described SCs demonstrated a further possibility that the geometry of the active site of the 'primitive' SCs has been already "prepared" for other cyclization products.

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CYCLIC DERIVATIVES FROM LINEAR MONO OR BIMETALLIC REAGENTS

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Metal-carbocyclization is a powerful method for creating cyclic organometallic derivatives. Among the many methods available presently, the carbolithiation according to Bailey *et al* is the most efficient one :

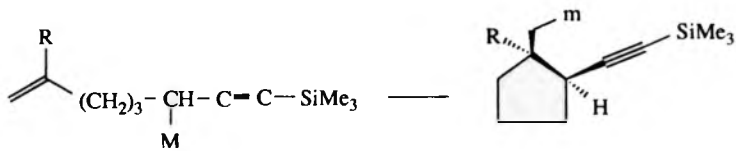
However limitations are observed according to the size of the ring (optimum C_5), the degree of substitution of the lithiated carbon : primary RLi >> secondary or tertiary, and the degree of substitution of the C=C bond.

We shall discuss the case of organozinc reagents, bearing a C=C bond δ to the metallated carbon, keeping in mind that organozinc reagents allow the presence of a variety of functions on the skeletton.

It turns out that primary zinc reagents cyclise efficiently, but also that secondary ones are even more prone to generate diastereoselectively substituted cyclopentyl methyl zinc reagents, contrary to the case of the corresponding lithium derivatives.

The stereochemistry is explained by considering an early or late transition state.

In the case of propargylic metallated systems, no cyclisation takes place with M = Li or MgX, but the corresponding zinc reagent cyclises smoothly within a few minutes.



We interpret these reactions via a metal ene-allene rearrangement, which explains the observed stereoselections. We shall discuss the case of secondary and tertiary propargylic zinc derivatives, and the use of such cyclisations for an approach to triquinane targets.

Finally internal addition of δ acetylenic organozinc reagents will be addressed.

SYNTHETIC APPROACHES TO SESQUITERPENOIDS WITH SEVEN-MEMBERED RINGS VIA [4 + 3] CYCLOADDITIONS OF OXYALLYL INTERMEDIATES.

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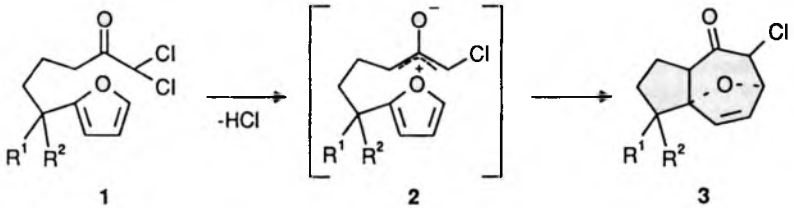
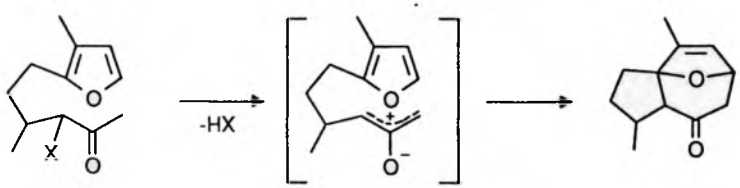
The [4+3] cycloaddition of oxyallyl intermediates derived from α-halogeno ketones to furans leads to 8-oxabicyclo[3.2.1]oct-6-en-3-ones. These oxygen-bridged seven-membered rings are investigated as precursors for the synthesis of sesquiterpenoids with a hydroazulene skeleton.
In persuit of our previous work on *intramolecular* [4+3] cycloadditions of oxyallyl intermediates [1,2], we have synthesized ketone precursors bearing a terminal furyl group, and explored the reaction with triethylamine in lithium perchlorate/diethyl ether. Cyclocondensations occur, leading to substituted hexahydro-8*H*-3a,6-epoxyazulen-8-ones. The dichloroketones **1**, prepared from furfural, formed the bridged hydroazulenes **3**, *via* chloro-substituted oxyallyl intermediates **2**.



1 **2** **3**

R^1, R^2 = CH_3, H (**a**); CH_2 (**b**); OCH_2CH_2O (**c**)

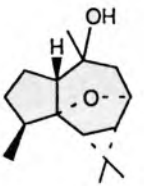
Starting from the commercially available (*Z*)-3-methyl-2-penten-4-in-1-ol, the α-mesyloxyketone **4** (X = CH_3SO_2O) has been synthesized. Treatment of **4** (X = CH_3SO_2O) with triethylamine in lithiumperchlorate/diethylether leads to oxatricyclic ketones **6**. Both **3** and **6** are formed as a mixture of diastereomers.



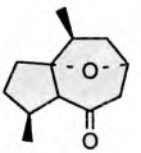
4 **5** **6**

Aiming at sesquiterpenes as goals, we have transformed the cycloadduct **3a** into further tricyclic intermediates, *int. al.* the tetracyclic aromadendrane derivatives **7α** and **7β**.

One of the diastereomeric cycloadducts **6** was hydrogenated with perfect *exo*-selectivity to give the saturated oxatricyclic ketone **8**.

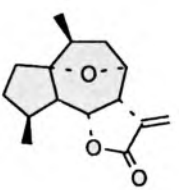


7α : α-OH
7β : β-OH

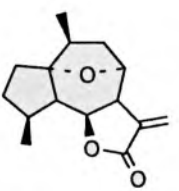


8

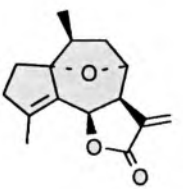
The Minato-Marshall annulation sequence [3], starting with an alkylation by methyl bromoacetate, afforded γ-lactones, which could be transformed into the corresponding α-methylene-γ-lactones **9a** and **9b**. These guaianolides are dihydro epimers of the natural sesquiterpene lactone 1α,8α-epoxyosmitopsin (**10**) [4].



9a



9b



10

Cyclocondensation of 2-(3-butenyl)-3-methylfuran [2] with pentachloroacetone affords 1-(3-butenyl)-2,2,4,4-tetrachloro-7-methyl-8-oxabicyclo[3.2.1]oct-6-en-3-one in good yield, by way of a tetrachloro-oxyallyl intermediate. This oxabicycle has been dechlorinated and transformed into (3aα,4β,6α-1,4-dimethyl-2,3,4,5,6,7-hexahydro-8*H*-3a,6-epoxyazulen-8-one, i.e. a dehydro derivative of ketone **8**. Annulation of the γ-lactone ring and methylenation eventually led to racemic **10** and two of its epimers.

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The Influence of Medium Acidity and the Mode of Carbocations Generating on the Choice of Predominant Route in Molecular Terpene Rearrangements. Experimental and Calculation Method of Prognosis

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Abstract not received

POSTERS

ECDYSTEROID BIOSYNTHESIS IN HAIRY ROOT CULTURES OF *Ajuga reptans*

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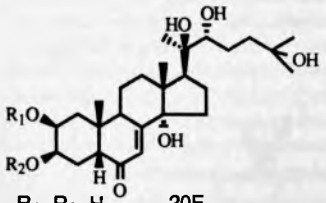
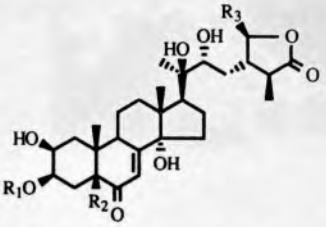
Diverse ecdysteroids have been identified in plants of the genus *Ajuga*. Among them, the depicted structures are present in methanol extracts of *in vitro* root cultures of different varieties of *Ajuga reptans*[1]. On the other hand, *A. reptans*, var, *atropurpurea* leaves inoculated with *Agrobacterium rhizogenes* produce "hairy roots" which exhibit a faster growing and higher ecdysteroids production, mainly of 20E, in comparison with wild type plants. For these reasons hairy roots are considered appropriate for ecdysteroid biosynthetic studies[2].

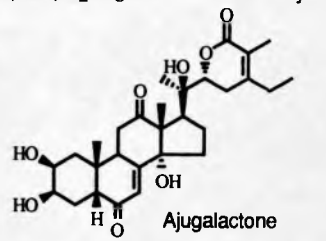
Our interest in ecdysteroids biosynthesis in *A. reptans* is focused on compounds with a cyclic side chain. In this communication, the obtention of hairy roots, by inoculation with *A. rhizogenes*, from different varieties of *A. reptans* and the changes induced on ecdysteroids composition will be reported. The most convenient transformant, in terms of C-28 and C-29 ecdysteroids production, was used for biosynthetic studies. Results on the incorporation and biotransformation of putative radiolabelled ecdysteroid precursors in this variety of *A. reptans* hairy roots will be presented. Finally, several conclusions suggesting possible biosynthetic pathway(s) for the different families of ecdysteroids produced in *A. reptans* will also be discussed.



$R_1=R_2=H$	20E
$R_1=Ac$; $R_2=H$	2-O-acetyl-20E
$R_1=H$; $R_2=Ac$	3-O-acetyl-20E



$R_1=R_2=H$; $R_3=Me$	Cyasterone
$R_1=R_2=R_3=H$	29NorCY
$R_1=H$; $R_2=OH$; $R_3=Me$	Sengosterone
$R_1=R_3=H$; $R_2=OH$	29NorSe
$R_1=Ac$; $R_2=H$; $R_3=Me$	3-O-acetyl-CY
$R_1=Ac$; $R_2=R_3=H$	3-O-acetyl-NorCY



Ajugalactone

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2. a) Nagakari, M.; Kushiro, T.; Yagi, T.; Tanaka, N.; Matsumoto, T.; Kakinuma, K.; Fugimoto, Y. *J. Chem. Soc., Chem. Commun.*, **1994**, 1761 and references cited therein. b) Delbecque, J.-P.; Beyron, P.; Chapuis, L.; Corio-Costet, M.-F. *Eur. J. Entomol.* **1995**, *92*, 301.

ECDYSTEROID BIOSYNTHESIS IN PROTHALLI CULTURES OF *Polypodium vulgare*

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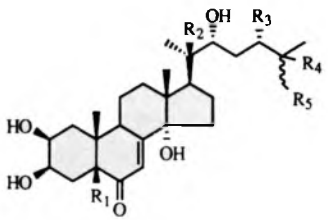
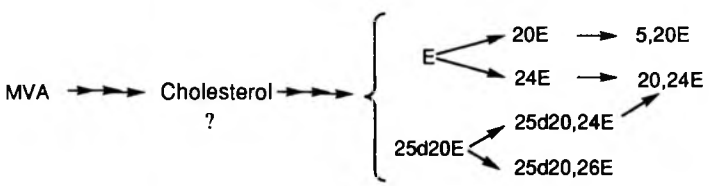
It is accepted that the contents of phytoecdysteroids depend on the plant part, the season and the habitat. For this reason, *in vitro* cultures are a widely used technique in plant research since they offer a mass production of secondary metabolites without seasonal or individual variations, and therefore being very useful for biosynthetic studies. *In vitro* cultures of small foliaceous structures (prothalli) produced from spores of the fern *Polypodium vulgare*, showed the presence 20-hydroxyecdysone as major ecdysteroid. In addition, seven minor compounds, i.e., ecdysone, polypodine B, inokosterone, abutasterone, 5-hydroxyabutasterone, 24-hydroxyecdysone and pterosterone, were also detected [1,2].



	R_1	R_2	R_3	R_4	R_5
Ecdysone (E)	H	H	H	OH	H
20-Hydroxyecdysone (20E)	H	OH	H	OH	H
Polypodine B (5,20E)	OH	OH	H	OH	H
Inokosterone (25d20,26E)	H	OH	H	H	OH
Pterosterone (25d 20,24E)	H	OH	OH	H	H
24-Hydroxyecdysone (24E)	H	H	OH	OH	H
Abutasterone (20,24E)	H	OH	OH	OH	H
5-Hydroxyabutasterone	OH	OH	OH	OH	H

Although the chemical structure of more than 100 phytoecdysteroids has been elucidated, heretofore little is known about their biosynthesis. In this communication, the incorporation and biotransformation of putative radiolabelled ecdysteroid precursors in prothalli cultures of *P. vulgare* will be reported. When radiolabelled ecdysone was applied to the prothalli all 25-hydroxyecdysteroids showed incorporation of tritium. In addition, mevalonic acid and cholesterol are bioconverted into ecdysteroids in a time dependent manner. From the results obtained the following pathway for the last steps of ecdysteroids biosynthesis in *P. vulgare* may be postulated.



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A NEW AND EFFICIENT SYNTHESIS OF 17α-HYDROXYCHOLESTEROL

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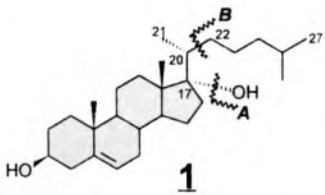
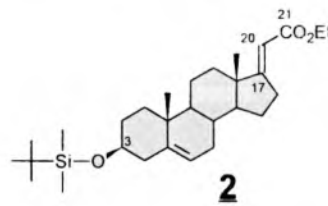
Previous syntheses of 17α-hydroxycholesterol (**1**) have involved oxidation at C-17 after establishing the full carbon skeleton (Strategy *A*). Our approach has been to elaborate the side chain on intermediates already containing the 17α-oxy function (Strategy *B*).



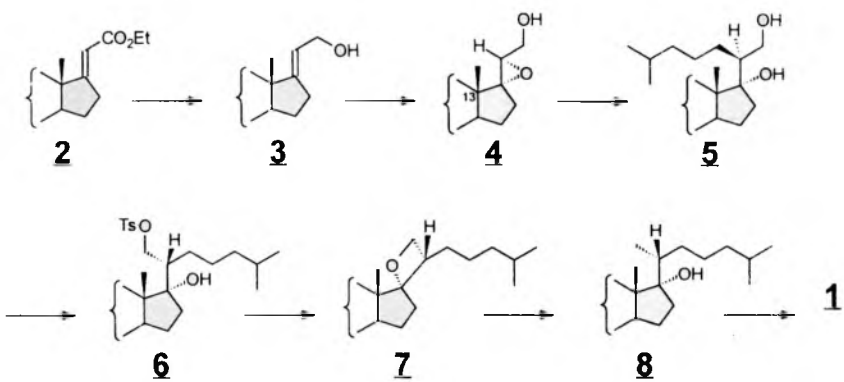
1



2

Building on the work of Wicha's group,[1] carbons 20-21 were incorporated using the stereoselective Wadsworth-Emmons reaction of the 3β-O-TBS-protected 17-keto steroid starting material, dehydroepiandrosterone, with triethyl phosphonoacetate. The remaining steps from **2** were envisaged to be: (a) chemo- and stereoselective epoxidation of the 17,20-double bond; (b) regio- and stereoselective opening of the resulting epoxide with a synthon for the C-22-to-27 residue of the side chain; and (c) conversion of the ester function, C-21, to a methyl group.

The successful route was as shown in the Scheme:



2 **3** **4** **5**

6 **7** **8**

In practice, the epoxidation step was carried out on the allylic alcohol (**3**), obtained after DIBAL reduction of the ester, using Sharpless' conditions [TBHP with (-)-DIPT] yielding the α-epoxide (**4**) selectively. This compound was prone to a Wagner-Meerwein-type rearrangement (with migration of the 13-methyl group) unless stabilised by base. The desired opening of the epoxide was achieved with isohexyl magnesium bromide under copper(I) catalysis to give the 17,21-diol (**5**). Deoxygenation of the primary (C-21) alcohol via LAH reduction of the tosylate was complicated by the fact that this proceeded in two stages: a fast spirooxetane formation followed by slow hydrogenolysis. The synthesis of **1** was completed by desilylation of **8**.

Some observations on the epoxidation step and other interesting reactions encountered in the above and modified strategies that did not come to fruition will also be discussed.

Reference: 1. Wicha J., Bal K., Piekut, S.: *Synth. Commun* 7, 215 (1977).

MODEL HAPTENS DERIVED FROM SOME AMINO STEROIDS

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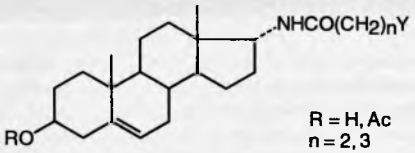
166 10 Prague 6, The Czech Republic

Steroid amines prepared from corresponding azides by a catalytic hydrogenation or nickel boride reduction were transformed into amides with hemi-esters of dicarboxylic acids or with 3-(p-hydroxyphenyl)propionic acid. Reactions of resulting model amides were studied to optimize the synthetic ways leading to haptens or tracers for testosterone or dihydrotestosterone immunoassays.



R = H, Ac
n = 2, 3
Y = COOH, p-HOPh

AN EXPEDIENT SYNTHESIS OF [16,16,17-$^2H_3$]-EPITESTOSTERONE VIA A ONE-POT DEUTERATION AND REDUCTION OF THE 17-KETONE FOLLOWED BY EPIMERIZATION OF α-DEUTERATED ALCOHOL

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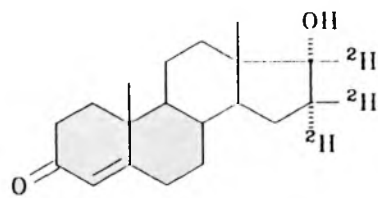
6β-Methoxy-3α,5-cyclo-5α-androstan-17-one was deuterated and immediately reduced by sodium in deuterium oxide to [16,16,17-$^2H_3$]-3β-methoxy-3α,5-cyclo--5α-androstan-17β-ol. The 17-tosyloxy group, in the corresponding derivative, was found to be stable under the conditions[1] of i-steroid rearrangement. The S_N2 reaction with potassium nitrite[2] of [16,16,17-$^2H_3$]-3β-acetoxy-17β-tosyloxy-5-androsten yielded, without the loss of deuterium, the epimeric [16,16,17-$^2H_3$]-3β-acetoxy-5-androsten--17α-ol, which was converted by hydrolysis and oxidation to the tittle labelled epitestosterone(I). The position deuterio atoms was confirmed by NMR[3] and isotopical purity was verified by MS[4].



[16,16,17-$^2H_3$]-EPITESTOSTERONE

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ISOLATION AND CHEMICAL TRANSFORMATION OF A NEW METABOLITE OF *FUSIDIUM COCCINEUM*

Welf von DAEHNE, Wagn Ole GODTFREDSEN
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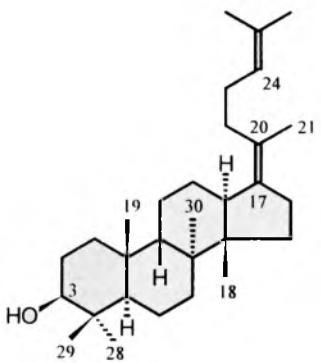
In the late 1960s, 3β-hydroxyprotosta-17(20)*Z*,24-diene (**1**) and 3β,29-dihydroxy-protosta-17(20)*Z*, 24-diene (**2**), common biosynthetic precursors of the triterpenoid antibiotics fusidic acid and helvolic acid, were isolated independently from the mycelia of the producing fungi, *Fusidium coccineum* [1,2] and *Cephalosporium caerulens*.[3-6]



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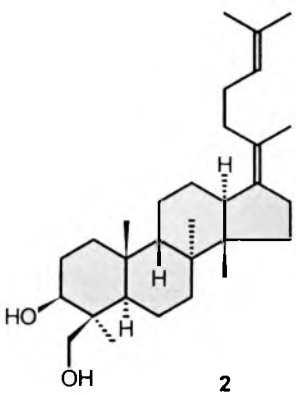

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Recently, a hitherto unknown metabolite of *Fusidium coccineum*, also produced during the industrial fermentation process, was isolated in substantial amounts by fractionation of the mother liquors from which fusidic acid had been separated. The new metabolite could be readily transformed by chemical means into the known protosterol (**1**) and diol (**2**), as well as a number of related structures. The chemistry of the new compounds and their possible role in the biosynthesis of fusidic acid will be discussed.

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OLEFIN FORMATION FROM HEPTAFLUORBUTYRYL DERIVATIVES

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Hydroxy-derivatives are converted to olefins via, e.g. their tosylates or mesylates. Disadvantage of these sulfonates is their instability.

We have found that hydroxy-compounds may be converted into correspomding esters - heptafluorobutyrates which are easily converted to appropriate olefins. The heptafluorbutyrates are very stable and may by stored for a long time without decomposition.

SYNTHESIS OF ESTRADIOL DERIVATIVES FUNCTIONALIZED AT C-6 BY SUBSTITUTED ARYL AND ALKYL GROUPS MEDIATED BY TRANSITION METALS

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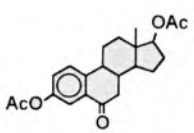
Interest in estradiol derivatives functionalized at C-6 by substituted aryl and alkyl groups can arise from special biologic activity or from usefulness in the preparation of bioassays.
The synthesis of these compounds proceeds usually via reaction of 6-oxo-estradiol derivatives **1**, obtained in moderate yield by benzylic oxidation of estradiol diacetate [1] with classic metalorganics like Grignard compounds [2] or the Reformatsky reagent [3]. Unfortunately, the benzylic oxo group is highly sensitive to enolization. Especially in the case of the strongly basic Grignard compounds enolization prevents the conversion into 6-substituted products in acceptable yields.
In contrast, we used *in situ* transmetallation to cerium-(III)-organyls [4] or a catalytic palladium intermediate [5] for effective 6-arylation and alkylation.
Enolization of the 6-oxo group is avoided with the weakly basic cerium-(III)-organyls leading to **2a** after treatment with acid in 85 %. In the case of palladium just that strong enolization tendency is utilized to generate the corresponding enetriflate which is smoothly converted in a next step into the 6-substituted product **4** in a catalytic Heck reaction. **4** is elaborated in 4 steps into the carboxylic acid **5** which is suitable for the preparation of radioimmuno assays.



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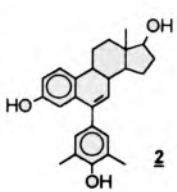

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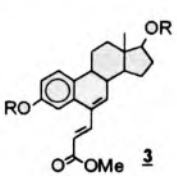

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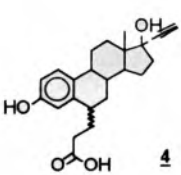

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CHIROPTICAL PROPERTIES OF STEROIDAL ALCOHOLS COMPLEXED WITH $[Rh_2(OCOCF_3)_4]$

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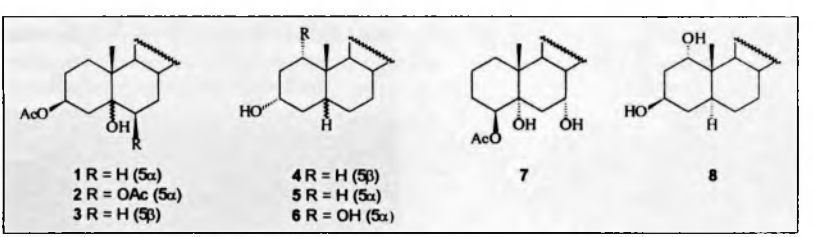
The absolute configuration of alcohols, synthetic or derived from natural compounds, can be determined by different methods. In 1990 Gerards and Snatzke[1] developed a new, fast and very useful method for this purpose. They have found that secondary monohydroxyalcohols ligate to the $[Rh_2(OCOCF_3)_4]$ cluster at the axial positions thus giving a new chiral complex. This complex, obtained *in situ* (hexane or chloroform) gives up to 5 CD bands in the 700-300 nm range. The sign of the band E observed at ca. 350 nm was unequivocally connected with the stereochemistry of the ligand. It was always positive (or negative) if the absolute configuration was specified as "b*S*" (or "b*R*").

Recent studies of some secondary alcohols of shugar series and α-hydroxy-esters[2] have shown that application of the Snatzke's rule to this class of compounds is not straightforward because it is sometimes extremly difficult to assign the substituents to the M or L type of bulkiness.

In this comunication we would like to present further results of CD studies on *in situ* complexes of steroidal monohydroxy- and dihydroxyalcohols with $[Rh_2(OCOCF_3)_4]$. The partial structures **1-8** of the cholestane derivatives and representative CD curves of alcohols **1**, **3**, **5** and **8** are presented in Fig. 1 and 2, respectively.

Figure 1



1 R = H (5α)
2 R = OAc (5α)
3 R = H (5β)

4 R = H (5β)
5 R = H (5α)
6 R = OH (5α)

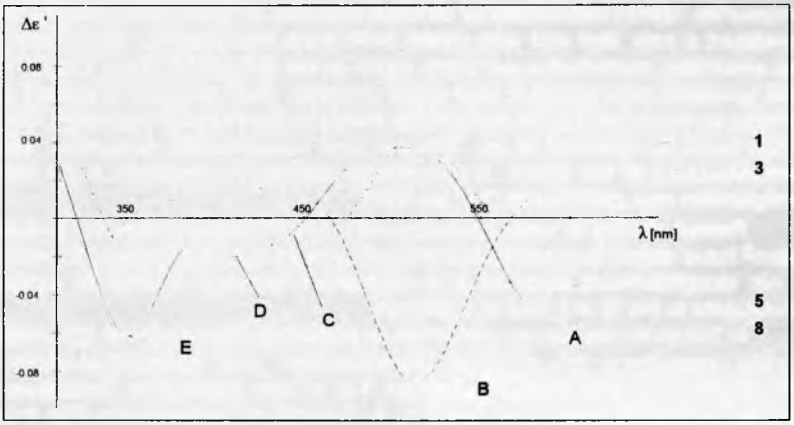
7

8

We have found that besides the secondary, also tertiary alcohols (e.g. compounds **1-3**) form optically active *in situ* complexes with the rhodium cluster. The negative CD band E is characteristic to 5α-steroids **1** and **2**, whereas 5β-hydroxy-steroid **3** shows positive CD band in the same region. All 3α-hydroxysteroids **4-6** (secondary alcohols) give positive CD band E independently of type A/B ring junction and the presence or absence of 1α-hydroxy group. Thus the complexation takes place probably through the oxygene atom at C-**3**. According to this, diol **8** (diastereoisomer of diol **6**) shows the negative band E Cotton effect due to the

reversed configuration at C-3. The secondary-tertiary diol **7** exhibits negative CD band E and forms complex exclusively through the less sterically hindered 7α-hydroxy group. For this compound we have succeeded in obtaining the chiral $[Rh_2(OCOCF_3)_4(diol\ \mathbf{7})_2]$ complex in a crystalline form. The structure of the adduct has been determined by the X-ray diffraction.[3] From X-ray data we have obtained an information about the complexing mode of alcohols with tetrakis(trifluoroacetato)-dirhodium (II). As in the case of olefines monohydroxyalcohols also form 1:2 adducts (diol : cluster) with $[Rh_2(OCOCF_3)_4]$.[4]

Figure 2: CD curves of complexes of alcohols **1**, **3**, **5** and **8** with $[Rh_2(OCOCF_3)_4]$.



The obtained results demonstrate an extention of validity of the Snatzke's *in situ* method to the tertiary monohydroxyalcohols. However, in the case of compounds with two hydroxy groups: secondary and tertiary, only the secondary one forms a chiral complex. In addition, it is necessary to note that the applicability of the Snatzke's bulkiness rule, relating sign of the Cotton effect E and the configuration of alcohol, for secondary and for tertiary alcohols is not satisfactory yet, and needs some further studies.

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RADICAL CYCLIZATION TO 3'-ALKYLIDENE-17-SPIRODIHYDROFURAN-SUBSTITUTED STEROIDS OBSERVATION OF AN UNEXPECTED INTRAMOLECULAR HYDROGEN TRANSFER

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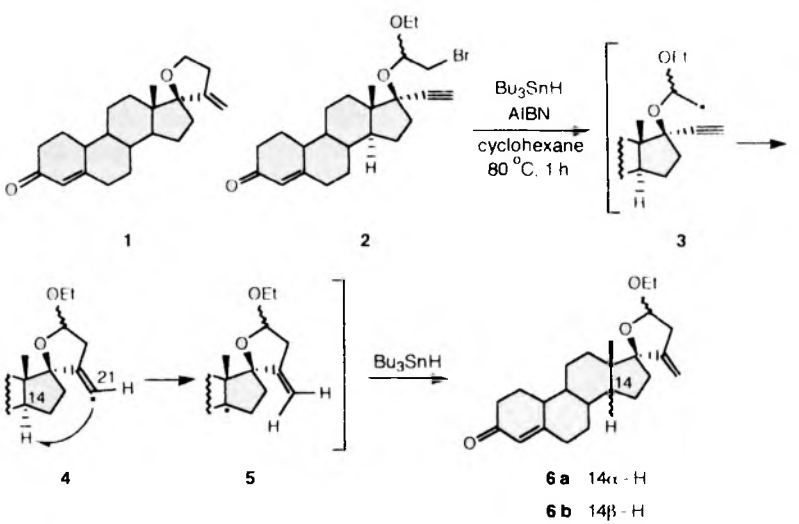
3'-Alkylidene-17-spirodihydrofuran-substituted steroids **1** form a new class of compounds having strong progestagenic properties [1]. Searching for methods of preparing these compounds we investigated whether bromo acetal **2** could be converted, via radical cyclization, into the spiromethylene compound **6a**. The cyclization product was obtained in almost quantitative yield (97 %), however ring closure was accompanied by complete inversion of configuration at C-14, giving **6b**. This epimerization can be explained by assuming that in the initially formed cyclization product **4**, an intramolecular hydrogen transfer takes place, from C-14 to C-21, before hydrogen abstraction from Bu_3SnH can occur. The newly formed species (**5**) then abstracts a hydrogen atom from Bu_3SnH, however the hydrogen comes in β, not α. As a result the more stable *cis*-fused CD ring system is produced.



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SYNTHESIS A NEW SERIES OF 4-AZAANDROSTANES

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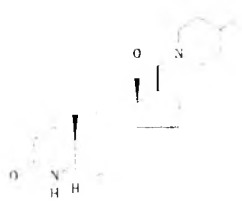
Benign prostatic hyperplasia is an androgen-dependent disease which afflicts a large percentage of males over the age of fifty, and is usually treated by surgery. Dihydrotestosterone, a 5α-reduced metabolite of testosterone, has been implicated as a causative factor in the progression of the disease, largely through the clinical study of males who are genetically deficient in the dihydrotestosterone producing enzyme, steroid 5α-reductase. As a result, inhibition of this enzyme has become a pharmacological strategy for the treatment of benign prostatic hyperplasia as well as other dihydrotestosterone related disorders such as acne and male pattern baldness.

During the past decades, because of their valuable therapeutic potential testosterone 5α-reductase inhibitors have been the subject of active research world-wide.

Our research effort relates to the investigation of various structural classes of 4-aza-steroid type 5α-reductase inhibitors. The results of these studies, the comparative inhibitory potency of these compounds with finasteride, and the possible clinical potential of 5α-reductase inhibitors will be presented.



The economic synthesis of the most potent derivative 17β-(4-Methylpiperidin-1-ylcarbonyl)-4-aza-5α-androst-1-ene-3-one will be described and it will be illustrated the general scheme that was used for the preparation of a number of compounds of this series.

Attention will be paid to the derivatives which are candidates for radioimmunoassay.

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SYNTHESES OF SOME STEROIDAL THIAZOLIDINES

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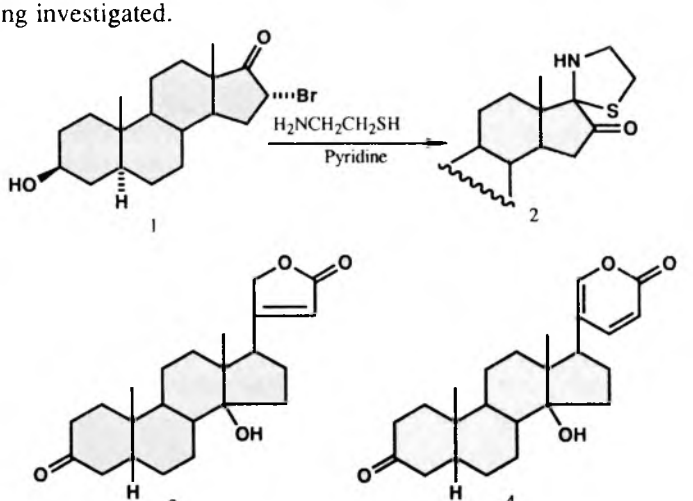
α-Keto spiro-2'-(1',3'-thiazolidine) deirvatives have been synthesized by the reaction of α-bromo steroidal ketones with 2-aminoethanethiol[1) 2)].

The reaction of 3β-hydroxy-16α-bromo-5α-androstane-17-one(1) with 2-aminoethanethiol yielded exclusively one of the isomeric 3β-hydroxy-5α-androstane-16-one -17-spiro-2-(1',3'-thiazolidine) derivative(2). It is shown that the formed product is probably the 17S-isomer.

Similar reaction of 2-aminoethanethiol with 3-ketodigitoxigenin(3-oxo-14-hydroxycard-20(22)-enolide)(3) and also with 3-ketobufalin(3-oxo-14-hydoxybufa-20,22 dienolide)(4) yielded mixtures of the 3S- and 3R-isomers of the 3-spiro-2'-(1',3'-thiazolidine)-14-hydroxycard-20(22)-enolide and 3-spiro-2'-(1',3'-thiazolidine)-14-hydroxybufa-20,22-dienolide in a 2:1 ratio. It is shown that the isomer accounting for two thirds of the reaction product is the more stable 3S-isomer. Biological effects of these thiazolidine derivatives are being investigated.



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STEROIDS AS A SCAFFOLD IN NON-PEPTIDE LIBRARIES

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Recently, Kalvoda[1] used a naphthalene derivative as a scaffold for the construction of protein ß-turn mimics, later for the same purpose Hirschmann[2,3] employed a disubstituted steroid derivative. Now we[4] have conceived the use of a steroid scaffold for the generation of non-peptide[5,6] libraries which would contain a rigid nucleus with three amino or pro-amino groups and a spacer for binding the molecule to a support. It was cholic acid which was chosen as the most convenient starting material. Its synthetic potential was recognized by Hirschmann[2] who pointed out two major reasons for the choice: the well known rigidity of the steroid molecule, securing distinct reactivity of individual functional groups, and the wealth of accumulated knowledge of partial transformations of individual hydroxy groups in the classical steroid literature.

Cholic acid was converted to 12α-(N-Allyloxycarbonyl)amino-7α-(N-t-butoxy-carbonyl)-amino-3-oxo-5ß-cholanic acid, its ^{1}H NMR and IR spectra verify that both amine protecting groups survived all reaction conditions used. The product was bound to beads through the carboxyl and then the third amino group was analogously created in the position 3.

Preliminary results, showing that non-peptide libraries based on this scaffold yielded compounds with useful activity, will be published elsewhere.

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REGIOSELECTIVE KETALIZATION OF 3,17-DIKETO-4,9-ESTRADIENE VIA IN SITU GENERATED OPEN CHAIN 3-KETALS

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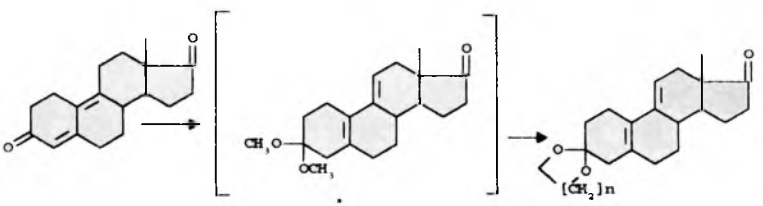
Regioselective ketalization of different keto groups in one molecule is a simple way for protection . It is a prerequisite for the functionalization of the remaining molecule especially on the non-protective keto groups.

While saturated ketones under ketalization conditions react according to uniform foreseeable rules, a complex reaction behaviour is observed in α,β- and $\alpha,\beta,\gamma,\delta$-unsaturated ketones. This is attributed to the neighbouring olefinic double bonds which affect the reactivity of the keto-group and vice versa. For example an isomerization of double bonds simultaneously can occur with the ketalization.

Starting from 3,17-dioxo-4,9-estradiene, cyclic 3-ketals have been prepared in the presence of 1,2- and 1,3- diols via in situ generated 3,3-dimethoxy-5(10),9(11)-estradiene-17-on.



A high regioselectivity could be realized with 1,3-diols under kinetic control /1/.

-Different reaction mechanisms and reaction rates have been used to get advantages in selectivity.

Sterically voluminous 1,2- diols can react under thermodynamic control to the cyclic 3-ketals.

-The thermodynamic aspects were taken into consideration by calculation of educts and products. Structural and sterical aspects, for instance the internal strain of different cyclic ketones, the reactivity of open chain and cyclic ketals and the volume of the alcohol component were studied with respect to optimize the regioselectivity of the ketalization.

The different possibilities of applicability of these reactions will be discussed!

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HIGHLY STEREOSELECTIVE SYNTHESIS OF 20-(HYDROFURYL)STEROIDS *via* ISOXAZOLE DERIVATIVES

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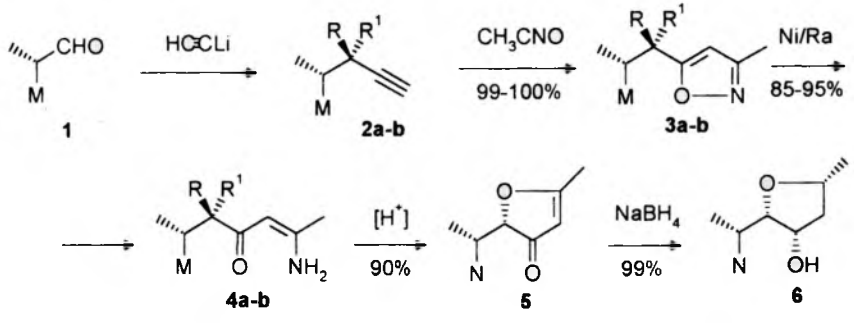
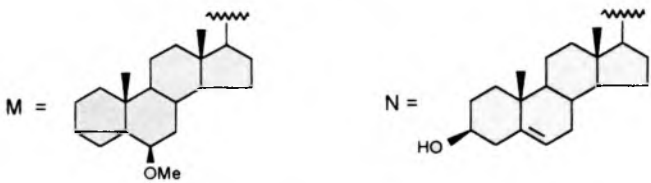
Cycloaddition reaction of steroidal acetilenic alcohols **2a-b** with acetonitrile oxide has been studied and efficient transformation of the adducts - 3,5-disubstituted



a: R=H, R^1=OH; **b**: R=OH, R^1=H



isoxazoles **3a-b** into 20-(hydrofuryl)steroids **5-6** has been realized *via* open-chain functionalized derivatives **4a-b**. Their further transformations proceeded highly stereoselectively allowing to obtain dihydrofuranone **5** as a single product. both from **4a** and **4b**. The sodium borohydryde reduction of **5** gave tetrahydrofuranol **6** in almost quantitative yield.

BRASSINOSTEROIDS WITH ESTER FUNCTION AT THE POSITION 20

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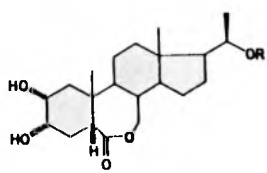
Brassinolide is a steroid compound with a seven-membered lactone ring in the skeleton [(22R,23R,24S)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-on]. Since the discovery of brassinolide, about thirty of its analogues have been identified in plants. The syntheses of brassinolide, its precursor (e.g. castasterone) and many other analogues is described in the literature[1]. However, the synthesis of brassinolide and other naturally occurring brassinosteroids are complicated and expensive. It is important for practical application of these compounds that these compounds have to be active and relatively easily available. In general, there are two possible approaches to the problem. The first one consits in attempts to improve synthesis to be more convenient for practical use, i.e. to be cheaper and more simple, the second one consists in the search for new active brassinosteroids with more simple structure. However, no compounds which would be applicable in a large scale have been obtained up to now. The reason is either the poor availability or a low activity or both of them.

The synthesis of pregnane analogues of brassinolide with an ester functionality in position 20 will be described and their activities in the bean second internode bioassay will be mentioned.



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HYDROBORATION OF 3-METHOXY-ESTRA-1,3,5(10),8,14-PENTAEN-17-OLS: A STEREOCHEMICAL INVESTIGATION

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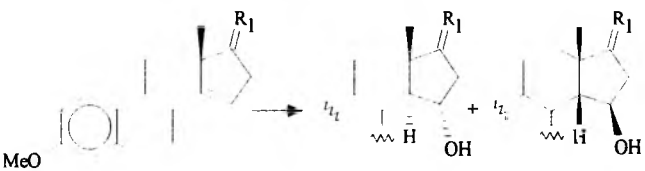
In our studies of the hydroboration of A-ring aromatic steroids we found two monohydroboration products of **1** upon alkaline hydrogen peroxide oxidation. **3** shows an unexpected stereo-chemistry in the light of the directive influence of the angular methyl group. We observed that **2** was transformed into **3** in the course of hydroboration. **3** respresents the thermodynamically more stable isomer.

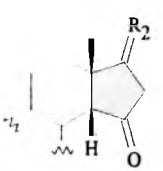


1, 4, 6, 8 **2** **3, 5, 7, 9**

R_1: **1, 2, 3** : α-H; β-OH
4, 5 : α-OH; β-H
6, 7 : α-H; β-OZ
8, 9 : α-OZ; β-H
Z=THP;Me

Our investigations were focused on two aspects: the influence of the position of the hydroxy group at C-17 and the effect of protective groups at this moiety. If **4** was hydroborated, we only obtained **5** as product. The product of an α-side approach (corresponding to **2**) was not detected. If the 17-hydroxy group was protected as THP or methyl ether (**6**, **8**), only products with a β-stereochemistry (**7**, **9**) were obtained. All these compounds showed a cis-C/D junction. Oxidation of 7 and **9** yielded the corresponding ketones; the THP ethers were deprotected upon oxidation. The 15-ketones **10** and **11** were investigated by CD measurement to confirm the 14β-stereochemistry.

Furthermore, we observed that the $\Delta^{8(9)}$-double bond was not completely inert for hydroboration. Bishydroboration products could be isolated. Their structures will be discussed.



10, 11
R_2:**10**:α-H;β-OY
11:α-OY;β-H
Y=H;Me

HYDROBORATION OF 3-METHOXY-ESTRA-1,3,5(10),8(14)-TETRAEN-17β-OL

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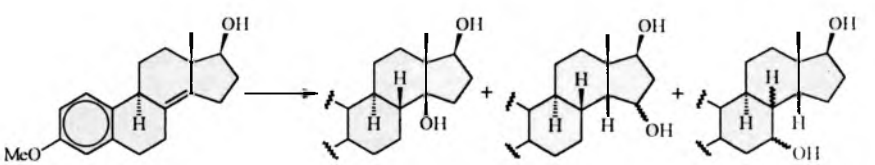
The hydroboration of 8(14)-olefins in the cholestane and ergostane series requires a higher temperature.[1] Contrary to this, the hydroboration / alkaline hydrogen peroxide oxidation of the aromatic steroid olefin **1** at temperatures lower than 40°C was reported to give **2** which is a key intermediate in the total synthesis of cardioactive steroids.[2] This reaction involved the formation of a number of unidentified products with different stereochemistry. Consequently, compound **2** was isolated in 20% yield only.

In the course of our studies of the hydroboration of double bonds present in aromatic steroids, we have reported very recently on the hydroboration of 6- and 7-dehydro estradiol-3-methylether. The results proved to be different from the reaction with the corresponding double bonds in non-aromatic steroids.[3]

In order to get a better insight into the electronical and / or sterical influence of the aromatic A-ring upon the hydroboration of steroid double bonds, the hydroboration of compound **1** was reinvestigated. We will report here that the 8(14)-double bond of **1** was attacked by borane from the front and the rear face of the molecule as well, followed by predominant isomerization of the initially formed steroidal boranes to the less hindered positions 7 and 15 of the steroid nucleus.[4] In accordance with the results in the cholestane and ergostane series, we isolated two 15-hydroxylated compounds (**3**, **4**) upon alkaline hydrogen peroxide oxidation. In addition, we obtained the unnaturally configurated 7-hydroxy steroids **5** and **6**. The product composition was dependent on the reaction temperature. The mechanism of the hydroboration will be discussed.



1 **2** **3**: 14α;15α **4**: 14β;15β **5**: 7α;8α;14α **6**: 7β;8β;14β

References:

1) Mincione, E. and Feliziani, F.: *J. Chem. Soc., Chem. Comm.* 942-943, (1973).
Mincione, E. and Feliziani, F.: *Ann. Chim. (Rome)* 65, 209-223 (1975).
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4) Brown, H. C.: *Organic Synthesis via Boranes*, Wiley Interscience, New York, 1975, pp. 77-78, and references cited therein.

SYNTHESIS OF STEROIDAL SALICYLIDENEIMINE LIGANDS AND THEIR COPPER(II) COMPLEXES FROM STEROIDAL AMINOALCOHOLS

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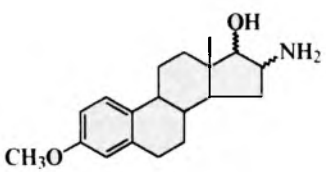
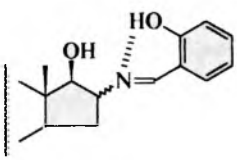
The aminoalcohols **1 a-d,** available from 3-O-methylestrone, were used as model compounds for metal complexation.

For example the condensation of **1a** and **1b** with salicylaldehyde furnished the salicylideneimines **2a** and **2b**.

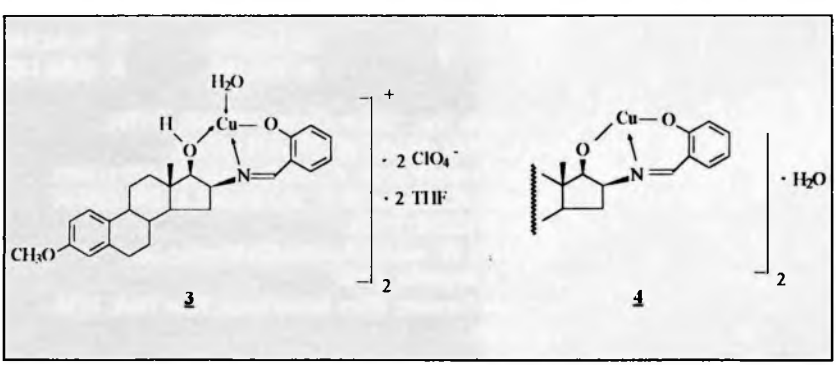


1 a : 16 β , 17 β
b : 16 α , 17 β
c : 16 β , 17 α
d : 16 α , 17 α



2 a : 16 β
b : 16 α

The reaction of this *Schiff*-base type ligands with copper(II) salts gave dimeric copper(II) complexes. This was proved by X-ray analysis for **3** and **4**. These complexes were obtained by mono- or dideprotonation of **2a**.



ONE-POT ESTERIFICATION AND SELECTIVE ACETYLATION OF CHOLIC AND DEOXYCHOLIC ACID

Ksenija KUHAJDA, Julijan KANDRAČ, Vera ĆIRIN-NOVTA, Dušan MILJKOVIĆ
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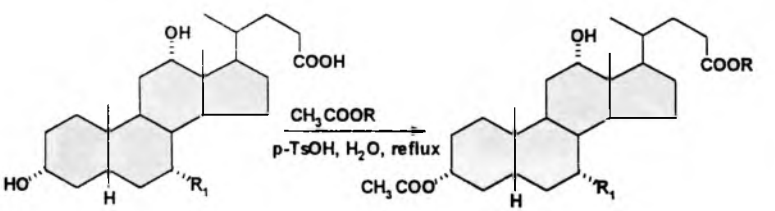
It is well known that cholic and deoxycholic acids 1 i 4 from ox bile are the major precursors for the commercial production of corticosteroids. In reported syntheses, esterification of carboxyl group of the side chain and selective acylation of 3α-hydroxyl group of bile acid are frequently used as the first and second step in these transformation processes.

The present work describes the one-pot esterification and 3α-acetylation of cholic and deoxycholic acids with different esters of acetic acid (ethyl acetate, propyl acetate and butyl acetate) and p-toluenesulphonic acid as catalyst.



1	R_1 = OH	R_1 = OH	R = CH_2CH_3
2	R_1 = OH	R_1 = OH	R = $(CH_2)_2CH_3$
3	R_1 = OH	R_1 = OH	R = $(CH_2)_3CH_3$
4	R_1 = H	R_1 = H	R = CH_2CH_3
5	R_1 = H	R_1 = H	R = $(CH_2)_2CH_3$
6	R_1 = H	R_1 = H	R = $(CH_2)_3CH_3$

Scheme 1

The yields of 3 α-acetoxy esters (ethyl, propyl and butyl) of cholic and deoxycholic acids were 74 - 95%.

SYNTHESIS OF 22,23,25-TRIFUNCTIONALIZED STEROID SIDE CHAINS *via* ISOXAZOLINE DERIVATIVES

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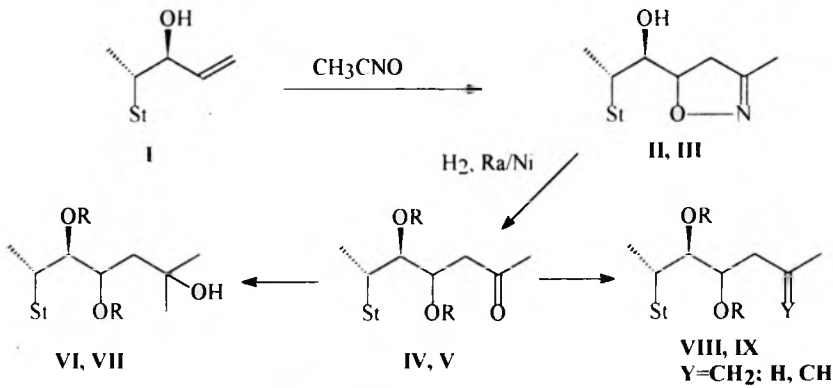
1,3-Dipolar cycloaddition of nitrile oxides to unsaturated compounds has been used by us in synthetic approaches to different side chains of natural steroids via adducts of some aliphatic nitrile oxides with 20(22)- and 22-ene steroids[1]. As a part of our research program in this area we have developped a route to polyhydroxylated side chain steroids starting from Δ^{23} -compounds.



II, IV, VI, VIII - 23R-isomer; **III, V,VII, IX** - 23-S-isomer R=H, Ac

It has been observed that the 1,3-dipolar cycloaddition of acetonitrile oxide to allylic alcohol **I** proceeded regio- and stereoselectively to give the mixture of epimers at C-23 **II** and **III**. Surprisingly, however, that the major isomer **II** (syn-isomer) had R-configuration at C-23. Thus the anti-directing effect of allylic oxygen substituent[2] is not presented here.

The possibility of selective transformation of the cycloadducts **II** and **III** to different open chain functional derivatives (**IV-IX**) gives a good perspectives for the employing of used methodology for synthesis a number of natural steroids such as brassinosteroids for example

Both the stereochemical aspects of cycloaddition of the spectral data of synthesised compounds will be discussed.

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2. Kozikowski A.P., Ghosh A.K. J.Org.Chem. 49, 2762 (1984)

SYNTHESIS OF A NEW STEROIDAL NEUROMUSCULAR BLOCKING AGENT

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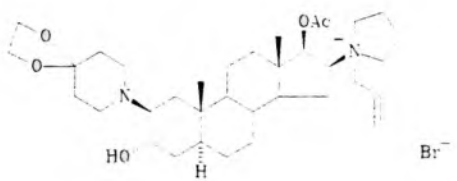
[1]Chemical Works of G. Richter Ltd., H-1475 Budapest, 10. P.O.B. 27., Hungary
[2]Montefiore Medical Center, New York 10467-2490, Bronx, 111 East 210th Street, USA

The steroid skeleton possessing onium centers at different position and configuration has given a good chance to study the structure—activity relationships within the series of neuromuscular blocking agents. In the last 30 years successful studies resulted in producing highly active drugs, pancuronium bromide, pipecuronium bromide, vecuronium bromide and rocuronium bromide.

In spite of these results a new nondepolarizing neuromuscular blocking agent with an even rapid onset, shorter duration and with faster recovery rate will be required in the future to replace the depolarizing suxamethonium. These, from the view-point of anesthetical practice contribute to controllability and safety of these compounds during surgical interventions.



In the course of our research work we have synthesized a new monoquaternary androstane derivative SZ—1677 {1-[17β-acetyloxy-2β-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-3α-hydroxy-5α-androstan-16β-yl]-1-(2-propenyl)pyrrolidinium bromide}, which has rapid onset, short duration and fast recovery of activity and good cardiovascular stability.

In our presentation we discuss the synthesis, the structure elucidation and the biological activity of SZ-1677 in comparison with the clinically already used neuromuscular blocking agents.

References:

[1] Z. Tuba et al.: HU Patent 210.076; European Patent Appl. 608.495.

[2] Földes, F. F.; Thut, P. D.; Cordes, C. T.: Comparison of the neuromuscular effects of SZ1676, SZ1677 and vecuronium in beagle dogs. Abstracts of 5th International Neuromuscular Meeting held in Tokyo, Japan, November 17-20, 1994. p 108.

SYNTHESIS OF SOME NEW D-HOMO-D-AZA ESTRONE DERIVATIVES

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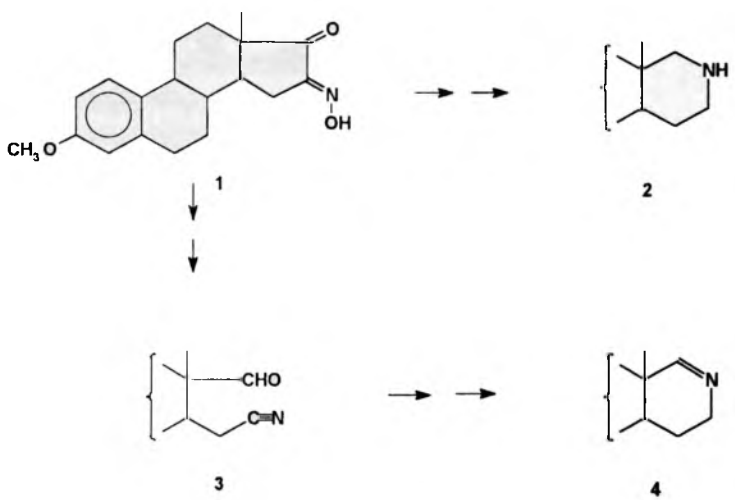
[2] *Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Yugoslavia*

D-aza derivatives of natural steroids show different biological properties in comparison with starting compounds[1]. Among them, of particular interest are D-aza estrone derivatives, due to their potential hypolipemic action[2].

Two synthetic routes for obtaining D-homo-D-aza estrone derivatives are reported. Namely, starting from 3-methoxyestra-1,3,5(10)-trien-16-oximino-17-one 1, in two steps, 3-methoxy-17-aza-D-homoestra-1,3,5(10)-triene 2 was synthesized. Another D-aza derivative was synthesized, starting from 3-methoxy-17-oxo-16,17-secoestra-1,3,5(10)-trien-16-nitrile 3, which was obtained from compound 1 in two steps. For that purpose, the seco cyanoaldehyde 3 was transformed into its 17-ethyleneacetal, followed by reduction of the nitrile function with lithium aluminium hydride. Finally, the obtained 16-amino-17-ethyleneacetal was, under acidic conditions, transformed into 3-methoxy-17-aza-D-homoestra-1,3,5(10),17(17a)-tetraene 4.



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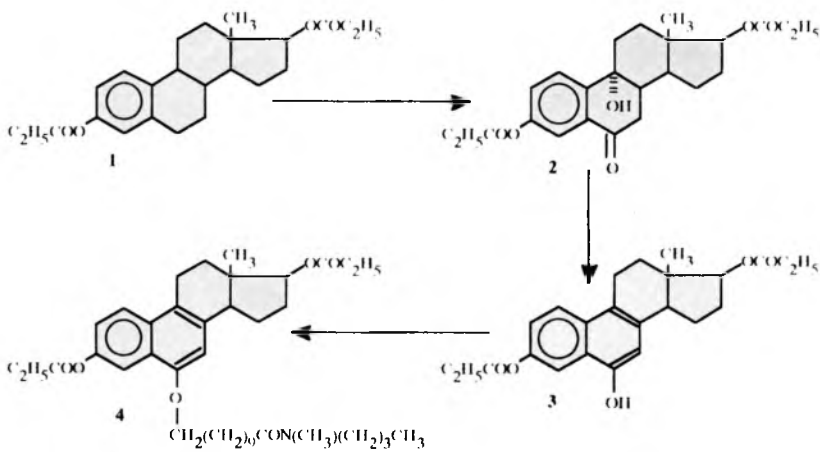
[2] Baran, J.S.,*J. Med. Chem.*, 1967, **10**, 1039

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 6-HYDROXY EQUILENIN DERIVATIVES

Marija N. SAKAČ, Dušan A. MILJKOVIĆ, Katarina M. PENOV-GAŠI, Julijana A. PETROVIĆ
Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Yugoslavia

Oxidation of estradiol dipropionate (**1**) by chromium trioxide-3,5-dimethyl pirazole complex afforded the 6-oxo-9α-hydroxy derivative **2**, which by action of thionyl chloride in pyridine underwent aromatization of its B-ring. Alkylation of the 6-hydroxy function of compound **3** with methyl-n-butylamide of 11-bromoundecanoic acid was achieved using tetrabutylammonium bromide in alcaline conditions.

Estrogenic and antiestrogenic activities of compounds **3** and **4** have been tested.



SYNTHESIS OF 2β-SUBSTITUTED CALCITRIOLS

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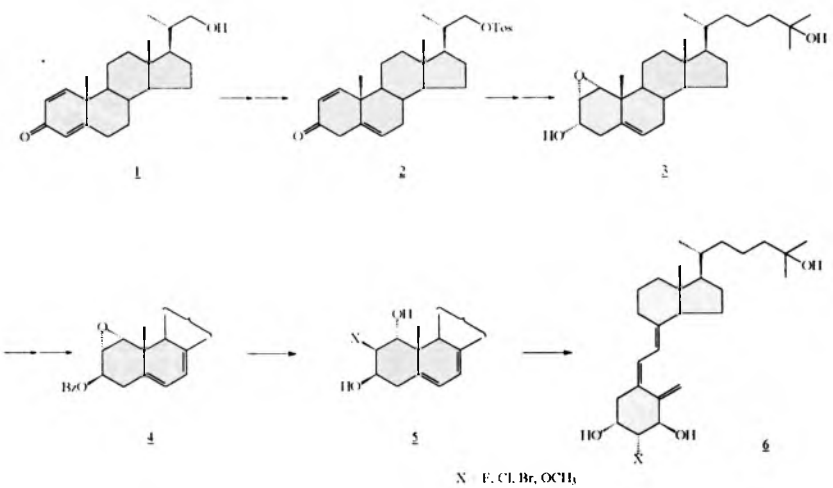
Starting with (20S)-20-Hydroxymethyl-pregna-1,4-dien-3-one (1) a number of 2β-substituted calcitriols was synthesized in eleven steps.



Key steps are the Selectride reduction of 2 and the titanium-catalyzed epoxidation of the 1-double bound after the side chain introduction. The 3β-benzoate 4 is available from compound 3 by Mitsunobu reaction and bromination/dehydrobromination. By cleavage of 4 with nucleophiles after saponification the provitamins 5 are obtained. Simultaneous irradiation with UV light of two wavelength, separation of the previtamins and thermal isomerization furnished the 2β-substituted calcitriols 6.

17-HYDROXY-14,15-METHYLENE STEROIDS

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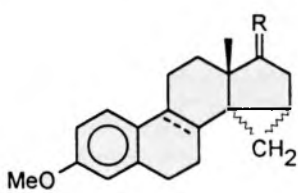
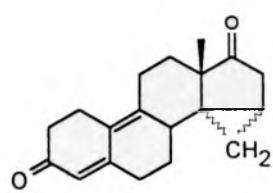
14α,15α-Methylene steroid **1**, an intermediate in the total synthesis of the strong estrogen 14α,15α-methylene estradiol (J 824)[1], was transformed into the dioxo dienes **2** and **3** which are potential precursors for novel antigestagens with a 14α,15α-methylene moiety. Key steps of this sequence were hydrogenation of the 8-double bond of **1** either by Birch protocol (Li, NH_3 / THF, $PhNH_2$ -50 °C), which gave compound **4** together with products formed by reductive cyclopropane ring opening, or by ionic hydrogenation (CF_3COOH, $HSiEt_3$, dichloromethane, 20 °C - 25 °C), affording **5** with a 14β,15β-methylene group. This cyclopropane ring inversion[2] was thought to be initiated by trifluoroacetic acid in an early stage of the hydrogenation. Indeed, **1** was shown to rearrange to **6** by p-toluenesulfonic acid in acetone or simply by thin layer chromatography on silica. Ionic hydrogenation of ketone **7** involved ring inversion also, leading to **8** which gave **5** stereospecifically upon borane reduction in the presence of (S)-1-(1'-amino-1'-phenylmethyl)cyclopentanol.
The structures were assigned by NMR-data and X-ray analysis. The circular dichroism of various 17-oxo 14,15-methylene steroids has been recorded.





1: R=17α-OH, 17β-H; 14α,15α; Δ^8
4: R=17α-OH, 17β-H; 14α,15α
5: R=17α-OH, 17β-H; 14β,15β
6: R=17α-OH, 17β-H; 14β,15β; Δ^8
7: R=O; 14α,15α; Δ^8
8: R=O; 14β,15β
9: R=O; 14α,15α
10: R=O; 14β,15β; Δ^8

2: 14α,15α
3: 14β,15β

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2. Cf. also: Künzer H., Thiel M.: Tetrahedron Lett. 35 2329 (1994).

NOVEL ESTROGENS WITH REDUCED METABOLIC BURDEN

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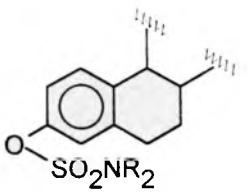
Otto-Schott-Strasse 15, D-07745 Jena, Germany
[2] En*Tec* Laboratories, D-07745 Jena, Germany

We report on the synthesis of sulfamates



R = H, alkyl, polymethylene

derived from ethynylestradiol, estriol, estrone, estradiol, and 14α,15α-methylene estradiol. After oral administration the compounds showed very high uterotropic activity in rats combined with a dramatic drop of the liver estrogenicity. The sulfamates are thought to circumvent first pass metabolism due to reduced sulfate conjugation or binding to blood proteins. Structure - activity relationships showed that in all studied series the unsubstituted sulfamates (R = H,H) had the highest activity and best dissociation as well. The esters may have potential use in fertility control and hormone replacement therapy. Clinical studies are under way.
We obtained the sulfamates by reaction of the corresponding sulfamoyl chlorides with the estrogens either in unprotected form (N-disubstituted esters) or upon protection of the D-ring hydroxyl groups as silylethers (N-mono- and N-unsubstituted esters). N-Disubstituted sufamates were available by PTC esterification. N-Methylsulfamates were formed in the presence of 2,6-di-*tert.*butyl-4-methylpyridine. Alternative bases (triethylamine, pyridine, DBU, DBO, DMAP) led to mixtures of the desired esters and products derived from reaction with N-sulfonylmethylamine. N-unsubstituted sulfamates were readily formed in the presence of triethylamine or upon reaction of the phenolic hydroxy group with sodium hydride in dimethyl formamide. To obtain the title compounds we developed selective methods to cleave the protective groups in the presence of the sulfamate moieties.

LONG RANGE EFFECT OF 17-SUSBTITUENTS ON HYDROGENATION 4,5-DOUBLE BOND

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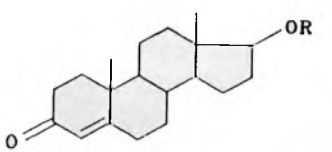
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Praha 6

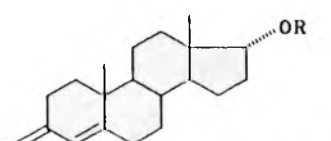
Long range effect of 17-substituent has been described for equilibration of 2ß,3ß-dihydroxy-6-oxosteroids'. Equilibrium constants are influenced by substituents in the position 17 ($K_{5\beta/5\alpha}$ = 0,56-7,06).

The long range effect on hydrogenation of the 4,5-double bond has not yet been systematically studied. There are some examples in the literature, e.g. hydrogenation of 3-oxosteroids with 4,5-double bonds afforded either exclusively or almost only 5ß-products[2-6]. However, some androstenedione, progesterone and testosterone derivatives[7,8] on hydrogenation afforded mainly 5α-isomers.

We desribe effect of 17-substituents on the ratio of 5α- to 5ß-products in hydrogenation of double bond of the 3-oxo-Δ^4-compounds in acetic acid on platinum catalyst. This effect was studied on series of esters, with an alkyl chain of different length, of testosterone (I) and epitestosterone (II)



(I)



(II),

where R = acyl group.

We have found that ratio 5α- to 5ß-products is influenced by substituent in the position 17 in great extent.

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SIMPLE DETERMINATION OF 5α/5ß-CONFIGURATION OF STEROID 3-KETONES

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Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo Nám. 2, 166 10 Praha 6, Czech republic

Determination of configuration at C_5 of 3-oxosteroids has been usually made on the basis of their circular dichroismus (CD) spectra: Cotton effect of 5α-3-oxosteroids is positive, Cotton effect of 5ß-oxosteroids is negative[1].

We have now found that the configuration at C_5 can be determined very simply from ^{1}H-NMR spectra. ^{1}H-NMR spectra of all studied 5ß-isomers (I) contain a triplet of one proton at 2,7 ppm. No such triplet was found at any of studied 5α-isomers (II). Examples and explanation of this effect will be given.

LITERATURE

1. Crabbé P.: "Saturated ketones and aldehydes", chap. II-7, p. 28, in "An Introduction to the Chiroptical Methods in Chemistry", Mexico 1971.

Biological status of brassinosteroids in insects

Karel SLÁMA[1] and Ladislav KOHOUT[2]

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Brassinosteroids - the growth factors of plants are polyhydroxylated steroids bearing some common structural characteristics with ecdysteroids - the moulting hormones of insects and other invertebrates. Almost 30 years ago, we have demonstrated that a number of derivatives related to 2,3-dihydroxy, 6-keto steroids (Hora et al.,1966; Velgová et al.,1968), which are now classified as derivatives of castasterone, had potential antiecdysone activity in insects. Later on, it has been shown that these effects were nonspecifically produced by general antisclerotization activity of these steroids in insects.

In a series of recent papers, derivatives of brassinosteroids or derivatives of 2,3-dihydroxy, 6 -keto steroids (castasterone) have been claimed to exhibit potential activity of antiecdysteroids, e.g. they antagonized the effects of ecdysone certain assays performed on fly larvae. In order to obtain more data in this respect, we have now subjected 13 derivatives of brassinosteroids and castasterone to standard bioassays for insect moulting hormone. In addition, selected brassinosteroids were subjected to competitive synergistic-antagonistic assays in the ligated larvae of the greater was moth (*Galleria mellonella)* and the meat fly (*Sarcophaga bullata).*

Unlike to some nonsteroidal ecdysone agonists (RH-5489), which can mimic the action of ecdysteroids, the 13 brassinosteroid derivatives investigated had positively no ecdysone activity whatsoever. Moreover, careful reinvestigation of possible antagonistic effects with 24-epibrassinolide and 24-epicastasterone revealed no effects on 20-hydroxyecdysone induced moulting process in both species of insects. Similarly negative antagonistic results were obtained with 2,3,17-trihydroxy, 6-keto androstane and the corresponding brassinolide derivatives. We conclude, therefore, that the reported effects on potential antiecdysteroid activity of brassinosteroids in insects are only illusive, as they cannot be confirmed by standardized bioassay techniques.

Hora, J. et al.,1966, Steroids 8:887-914; Velgová et al.,1968: Coll. Czech Chemn. Commun. 33:242-256.

NMR SPECTROSCOPIC STUDIES OF 3-METHOXY-14,15-METHYLENESTRA-1,3,5(10),8-TETRAEN-17-OL

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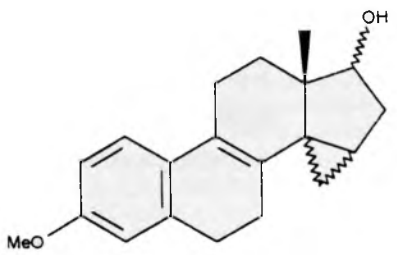
Herrmann et. al. [1] reported the influence of pyridine on the chemical shift of the 13-methyl group in dependence on the orientation of the 17-hydroxyl group. We used this effect for configuration studies of 14,15-methylentetraenols.



For quantification of these effects we used the differences of chemical shifts in spectra obtained in d_5-pyridine and $CDCl_3$. The shift differences of the 13-methyl groups are always 0.30 ppm for all 17β-hydroxy compounds. The reverse configuration of the hydroxyl group effects a different behaviour for 14α,15α-methylene-17α-hydroxy compounds and 14β,15β-methylene-17α-hydroxy compounds. As expect the former configuration shows only a small shift difference, the latter a difference of 0.19 ppm, nearly the value of 17β-configuration. This behaviour is easy to understand: the angle 13β-17α-position is usually 160-170°, in 14β,15β-methylene compounds the angle has a value of only 90°. That means interactions between pyridine and 13-methyl group are possible, resulting in the big shift difference.
The chemical shifts of the hydrogen atoms of the methylene bridge are very sensitive to the configuration of the 17-substituent. Using the same method described above the shift differences only depend on the cis or trans configuration of 17-substituent and bridge. This method is possibly usable for an easy analysis of C,D ring connections. Studies are under way.

LITERATURE

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NEW ECDYSTEROIDS FROM MUSHROOMS

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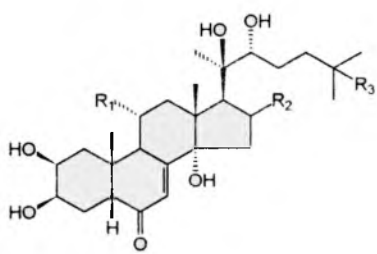
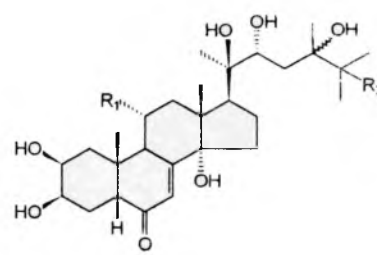
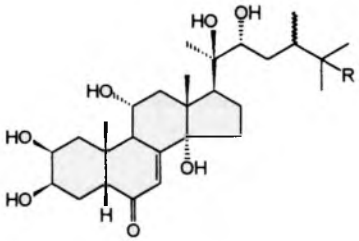
Ecdysteroids, compounds structurally related to the insect moulting hormone, ecdysone, are widely distributed in plant species belonging to a large set of families. Occurrence of some ecdysteroid-related compounds has been reported also in fungi (Mycophyta), however, their moulting hormone activity has not been described. This fact very probably retarded the investigation of ecdysteroids in fungi up to the recent time. The discovery of paxillosterone (**7**) from the mushroom *Paxillus atrotomentosus* (Batsch) Fr. in our laboratory initiated an extensive chemical prospecting in mushrooms. Screening of 78 selected species indicated that two of them contain ecdysteroids. Four cholestane-type ecdysteroids **1-4** were isolated from *Tapinella panuoides* (Fr. ex Fr.) Gilb., syn. *Paxillus panuoides* (Fr. ex Fr.) Fr. These compounds have already been known as plant constituents or snail metabolites. Additional three new ergostane-type ecdysteroids **5-7** were identified by the FAB-MS and NMR analysis. From *P. atrotomentosus* besides the major ecdysteroid paxillosterone (**7**), six new minor ergostane-type ecdysteroids **8-13** were isolated and identified. Most ecdysteroids from mushrooms are noted for the characteristic presence of 24-methyl and 11α-hydroxy groups in their structure.



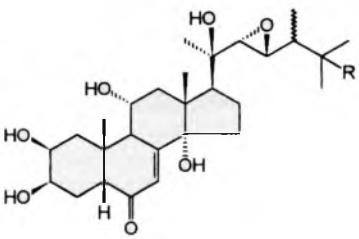


	R_1	R_2	R_3	
1.	H	H	OH	20-hydroxyecdysone
2.	H	H	H	ponasterone A
3.	H	OH	OH	malacosterone
4.	OH	H	OH	turkesterone

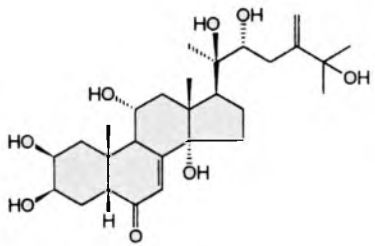
	R_1	R_2	
5.	H	H	panuosterone
6.	H	OH	25-hydroxypanuosterone
7.	OH	H	paxillosterone



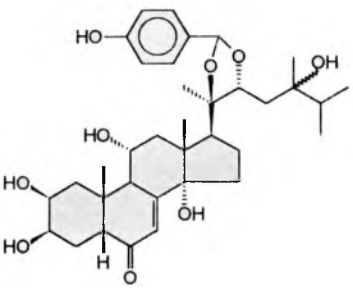
8. R = H atrotosterone A
9. R = OH 25-hydroxyatrotosterone A



10. R = H atrotosterone B
11. R = OH 25-hydroxyatrotosterone B



12. atrotosterone C



13. paxillosterone 20,22-*p*-hydrohybenzylidene acetal

REACTIONS OF STEROIDAL ENAMIDES WITH ELECTROPHILIC REAGENTS

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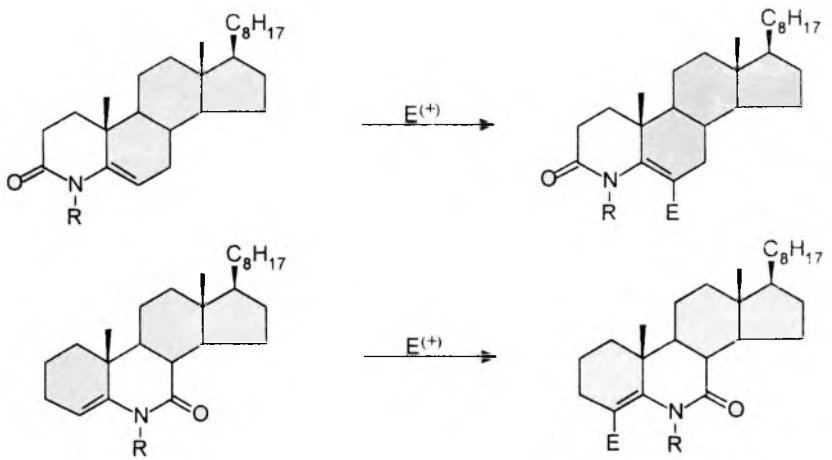
Two steroidal enamides, 4-azacholest-5-en-3-one and 6-azacholest-4-en-7-one, together with their N-methyl derivatives were prepared from cholesterol. The compounds were subjected to some electrophilic reactions: halogenation, nitration and oxidation.



It was established that the electrophiles ($X^{(+)}$ or $NO_2^{(+)}$) preferentially attack enamides at the terminal carbon atom (C-6 or C-4). No reaction at the nitrogen atom was observed . The further reaction took place at the allylic position. The CrO_3 oxidation resulted in the double bond cleavage to seco-acid (R=H) or in the hydroxyketone formation ($R=CH_3$). Some unusual by-products were formed presumably by radical mechanism. Reduction of the double bond in enamides with $NaBH_4/H^{(+)}$ was also studied. This reaction is preceded by protonation which catalyzes an equilibrium between enamide and acylimine forms.

SYNTHESIS OF NEW VITAMIN D ANALOGUES

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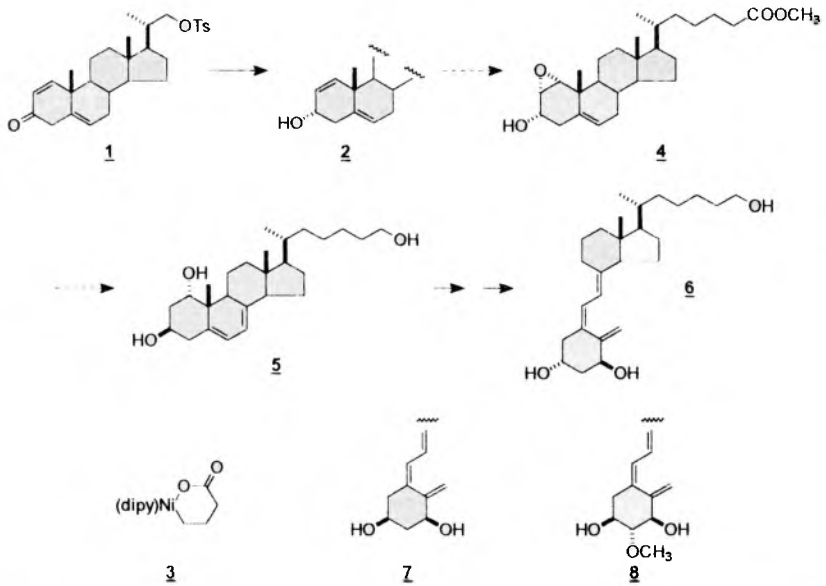
Using (20S)-20-Tosyloxymethyl-pregna-1,5-dien-3-one **1** as starting material the vitamin D analogue **6** can be synthesized in 11 steps.



Stereo- and regioselective reduction of **1** by LS-Selectride gives the 3α-hydroxy compound **2**. Side chain introduction by reaction of the 22-iodide with the nickel-alactone **3**, esterification and titanium catalyzed epoxidation of the 1-double bound lead to the 26-methylester **4**.

Provitamin analogue **5** is available after 3-epimerization by Mitsunobu reaction, bromination/dehydrobromination and oxirane cleavage by lithium aluminium hydride. The vitamin D analogue **6** can be obtained by simultaneous irradiation with UV light of two wavelengths, separation of the previtamin and thermal isomerization.

In a similar way 3-epivitamin **7** or 2β-substituted 3-epivitamin **8** are also available from the compound **2**.

NOVEL DIHOMOANALOGS OF 1,25-DIHYDROXYCHOLECALCIFEROL

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A novel synthetic method was developed for the preparation of side-chain extended analogs of 1,25-dihydroxycholecalciferol [1,25-dihydroxyvitamin D_3; 1,25-$(OH)_2D_3$]. This synthesis is a part of our search for the vitamin D analog with a selective activity profile and for further development as a candidate for the therapeutic agent. It has been demonstrated that the separation of both calcitropic and cell differentiation activity of vitamin D compounds might be obtained by synthetic modifications like the lengthening of the aliphatic side chain of the parent 1,25-$(OH)_2D_3$. The lead structure of this group of analogs (24a,24b-dihomo-1,25-$(OH)_2D_3$) showed in the *in vitro* studies the increased cell differentiation activity combined with the reduced or abolished major forms of calcemic activity. The binding affinity of the lead to the intracellular vitamin D receptor (VDR) was, however, two orders of magnitude lower than that for the endogenous 1,25-$(OH)_2D_3$. We now developed a novel synthetic method especially useful for the preparation of analogs of 1,25-$(OH)_2D_3$ with the side-chain extended by two or more carbon units. The method employs the use of the new vitamin D C_{25} synthon and an easily available side-chain fragment and represents an extension of our previously described strategy utilizing vitamin D C_{22} and C_{24} synthons. The key step of the present approach was the opening of oxirane ring of isobutylene oxide with the use of lithiated C_{25} vitamin D sulfone. Reductive desulfonylation and removal of the protecting groups gave the known lead identical with the authentic sample. The strategy based on vitamin D C_{25} synthon was also used for the preparation of new dihomoanalogs of 1,25-$(OH)_2D_3$. One of this compounds, quite unexpectedly, showed the binding affinity to VDR three orders of magnitude higher than that of other dihomoanalogs and ten times higher than that of 1,25-$(OH)_2D_3$.

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AN EFFICIENT SYNTHESIS OF 23-ENE-22-KETOSTEROIDAL SIDE CHAINS *via* ISOXAZOLE DERIVATIVES

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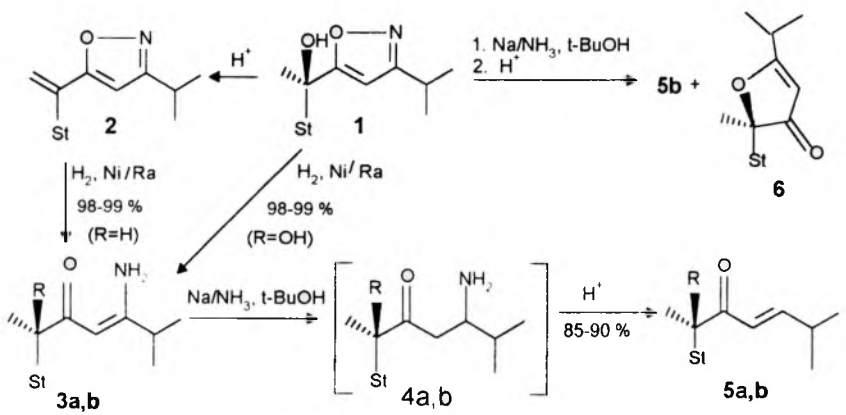
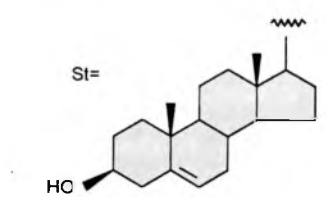
An efficient synthetic method for preparation of steroids with side chains containing α,β-unsaturated ketone moiety has been elaborated. The hydrogenation of steroidal isoxazoles **1, 2** over Raney nickel in ethanol gave the enaminoketones **3 a,b** in almost quantitative yields, and both of them further were transformed into α,β-unsaturated ketones **5 a,b** in high yields (85-90 %). This transformation includes the 23,24-double bond reduction in **3 a,b** by sodium in liquid ammonia followed by elimination of amino function of intermediate aminoketones **4 a,b** under acid conditions. Direct reduction of the isoxazole **1** by sodium in liquid ammonia followed by acid treatment gave the mixture of the ketone **5b** and the furanone **6** as major products (21 and 24 % respectively). The isoxazole **2** in similar conditions gave poor yield of the ketone **5a.**

The reaction conditions and spectral properties of synthesised compounds will be discussed.





a: R = H; b: R = OH

A NEW APPROACH TO THE CONSTRUCTION OF BRASSINOLIDE SIDE CHAIN

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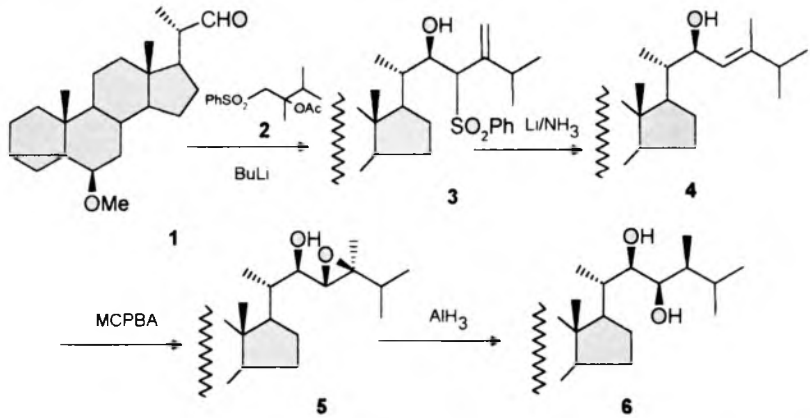
A new method of brassinolide side chain construction has been elaborated. It is based on the reaction of steroidal aldehyde **1** with the anion derived from β-acetoxy



sulfone **2** and butyl lithium. It was found that the reaction proceeded with formation of unsaturated sulfone **3**. Its reduction with lithium in liquid ammonia gave allylic alcohol **4**. It could be easily transformed into 22R,23R-diol **6** *via* epoxy alcohol **5**.

LIPASE-CATALYZED SYNTHESIS OF OPTICALLY ACTIVE TOCOPHEROL ANALOGS.

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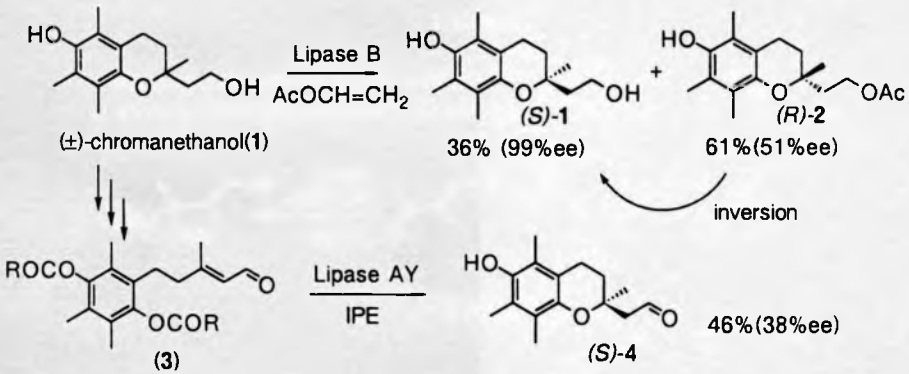
The chroman derivatives have various biological activities. Especially, these antioxidant activities are most important effect. Recently, many tocopherol analogs have been examined as antioxidants to search more effective hydrophilic scavengers of super oxide series such as Trolox and MDL-73404[1]. The chromanethanol is a key synthetic unit of these antioxidants of tocopherol analogs. We wish to describe in this conference, the synthesis of optically active chromanethanol by using the lipase-catalysis.

First, we investigated the lipase-catalyzed resolution of racemic chromanethanol(**1**) which was synthesized easily. From screening tests of enzymes, lipase B (Sapporo, *Pseudomonas fragi*) catalyzed esterification with vinyl acetate gave a enantiomerically pure *(S)*-chromanethanol as a recovered substrate in yield of 36%. And the *(R)*-**2** as a by-product was inverted to the *(S)*-isomer in several steps , that was then employed as a substrate of repeated lipase-catalyzed resolution.

Further methodology of synthesis of optically active chromanethanol was investigated and we found the lipases which could prepare the optically active chromanethanal[*(S)*-**4**] from the substrate αβ-unsaturated aldehyde(**3**). The substrate was synthesized from racemic chromanethanol in several steps. The enzymatic reaction in diisopropylether(IPE) at room temperature proceeded and gave the optically active chromanethanal[*(S)*-**4**].



C5-CHAIN ELONGATION AND FURTHER FUNCTIONAL GROUP TRANSFORMATIONS BASED ON CLAISEN REARRANGEMENTS.

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Department of Organic Chemistry, Royal Institute of Technology, KTH
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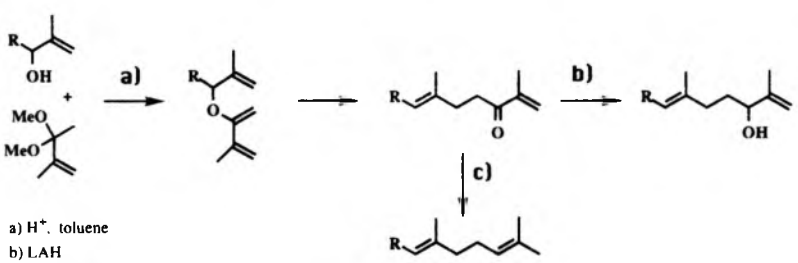
Claisen rearrangements, using the dimethyl acetal of methyl isopropenyl ketone and allylic alcohols followed by LAH reduction,[1,2] has previously been shown to be an efficient two-step iterative method for building head to tail isoprenoid chains. Termination of the chain, to the commonly occurring 2-methyl 2-propenyl group ("the fishtail ending") was accomplished by a one-pot deoxygenation with concomitant transposition of the double bond,[3] when applied to the α,β-unsaturated ketones, formed in the first step, see Scheme 1.



a) H^+, toluene
b) LAH
c) CH_3COOH, 1) p-$TsNHNH_2$, 2) $NaBH_3CN$
one pot

Scheme 1

In order to expand the utility of this approach we have now incorporated a vinylic bromide in the C5-unit. The synthesis of the new C5 building block is shown in Figure 1.

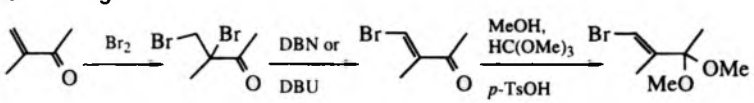


Figure 1

The Claisen rearrangement with the new building block proceeds in good yield. When the one pot reduction was applied to the resulting bromo enone two principal products could be obtained depending on reaction conditions. The *E*-methyl of the "fish tail ending" can thus be converted to an electrophilic carbon or a potential nucleophile as shown in Figure 2.

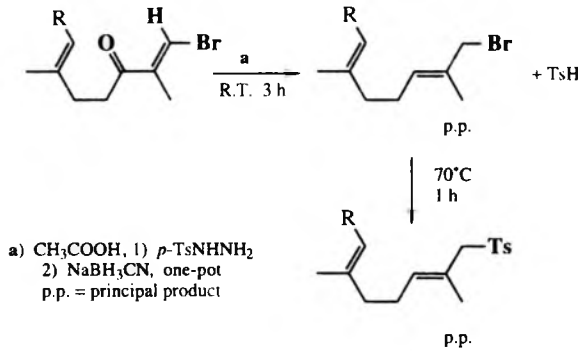


Figure 2

The allylic bromides were used as starting material for aldehydes by oxidation with *N*-methylmorpholine *N*-oxide as shown in Figure 3.

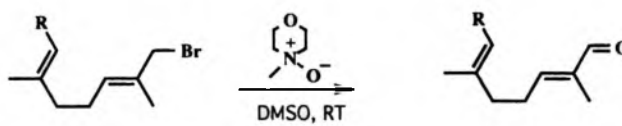


Figure 3

Details of the mentioned reactions in connection with synthesis of naturally occurring isoprenoids will be discussed.

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OPTICALLY ACTIVE ISOQUINUCLIDINE NITROXIDES FROM LIMONENE.

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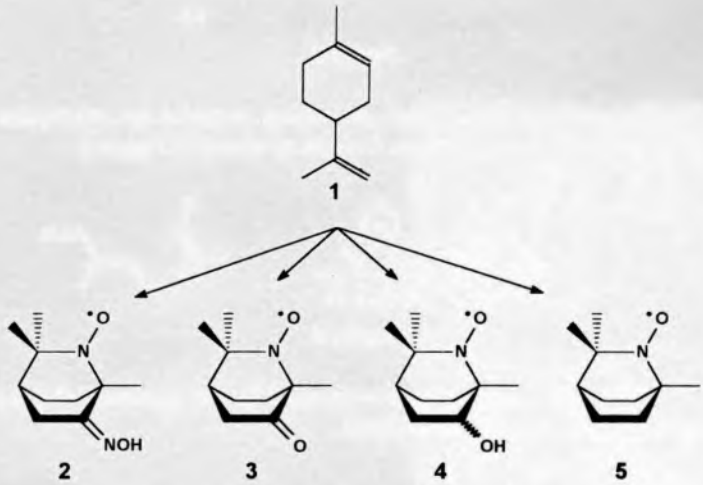
Stable free nitroxide radicals of diverse structural types have been prepared as spin labels to place recognizable molecules in many species of tissue, membranes, surfaces, etc., or as oxidizing agents. Among them some isoquinuclidine nitroxides have been described[1]. We have found that easily available limonene **1** is a convenient starting material for isoquinuclidine derivatives' preparation[2]. Limonene **1** may be easily transformed to the new α-hydroxylamino oxime, which is the precursor for a number of interesting bicycle free radicals **2-5**.



Globular structure of compounds **3-6** makes these optically active nitroxides a potential spin labels in biological systems or selective oxidizing agents.

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STRUCTURE OF ARTEFINE - A NEW SESQUITERPENE LACTONE FROM ARTEMISIA FILATOVII

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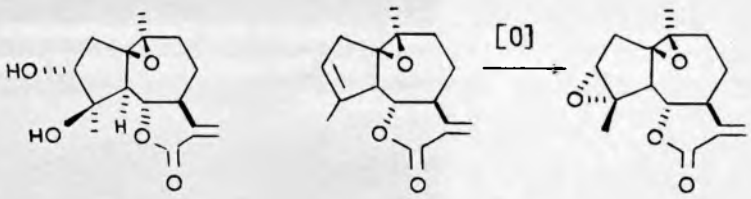
Scientific and Productional Introductional Small enterprise "Tabigat", Karaganda, Kazakhstan

From aerial part of *Artemisia Filatovii* there have been isolated two sesquiterpene lactones of guaiane type of which one was identified as isoepoxyestafiatin (3) and the second proved to be a new compound and was called as artefine(1).

Isoepoxyestafiatin is a colorless crystalline substance, $C_{15}H_{18}O_4$, m.p.167-170°C, $[\alpha]_D^{16}$ +49,9°(c 0,1;chloroform).We produced isoepoxy–estafiatin by epoxyding of sesquiterpene lactone arglabin(2) that had been isolated from *Artemisia glabella Kar.et Kir.*

At interaction of arglabin with per-acetic acid in methylene chloride there forms a mixture of two products,after separation of which by means of flash-chromatography on column with silicagel there have been isolated crystalline substances of which one proved to be identical to isoepoxyestafiatin.Yield is 5%.

Artefine is a crystalline substance, $C_{15}H_{20}O_5$, m.p.206-208°C, $[\alpha]_D^{16}$ +30,1°C (c 0,1;chloroform).The results of X - ray investigation of artefine monocrystal testify that (1) has a structure 3α,4β–dihydroxyisoepoxyestafiatine.



(1) (2) (3)

NEW LABDANE DITERPENES FROM *BRICKELLIA GLANDULOSA* (ASTERACEAE)

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The new world genus *Brickellia*, with about 90 species [1] is one of the largest taxa in the tribe Alomiinae (Eupatorieae) of the Asteraceae. Although the species are distributed from the Canadian border, southward through the western USA and Mexico and sparingly into central and southamerica; the greatest concentration is in the southwestern USA and Mexico.

We here report the isolation of seven labdane diterpenes from the flowers and leaves of *Brickellia glandulosa* , a member of the subsection Coleosanthus.

It is of interest to point out that most of the species studied so far, contained diterpenes of the labdane type [2].

Two collections of *B. glandulosa* were made; leaves from the plant collected before flowering yielded the compounds **1**,**3**,**4**,**5** and **7**, while the leaves from the plant collected after flowering yielded **1**,**4**,**5**,**6**,**7**. Finally, the flowers yielded only **1** and **2**.

The structure of the compounds were established from their spectroscopic data and their antibacterial activity were tested also. The compounds **5** and **7** were the more active against gram (+) and gram (-) bacteria.

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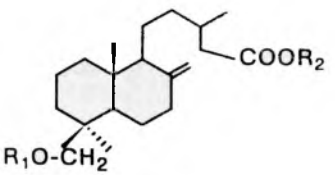
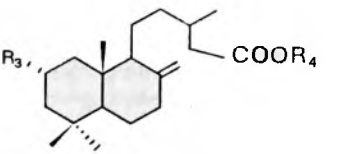


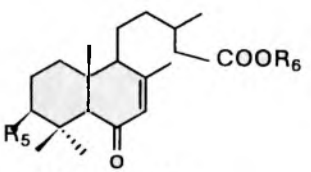
1.- R_1 = -CO-CH(CH_3)-CH2-CH3 R_2 = H

2.- R_1 = R_2= H



3.- R_3 = OAc R_4 = CH_3

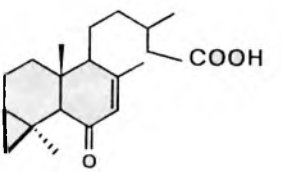
4.- R_3 = OH R_4 =H



5.- R_5 = R_6 = H

5a.- R_5 = H; R_6 = CH_3

6.- R_5 = OAc; R_6 = H

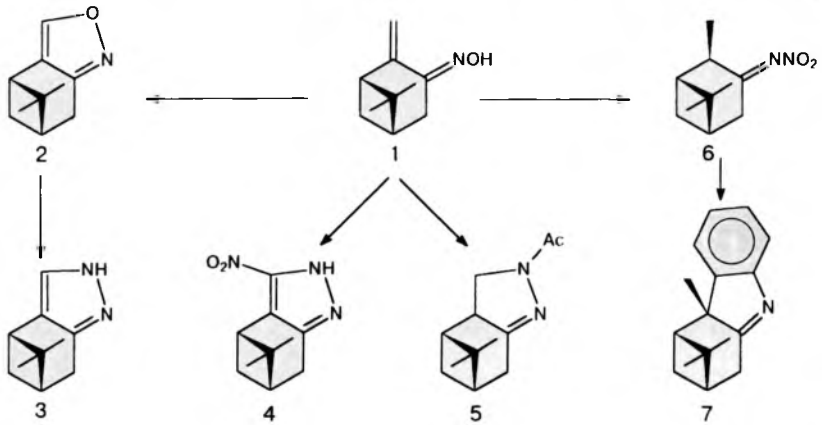


7

SYNTHESIS OF NITROGEN-CONTAINING HETEROCYCLES FROM PINOCARVONE OXIME.

Andrey M. CHIBIRYAEV, Sergey A. POPOV, Alexey V. TKACHEV
Novosibirsk Institute of Organic Chemistry, Acad. Lavrentjev ave. 9, Novosibirsk, 630090, Russia

α,β-Unsaturated oximes derived from terpene hydrocarbons are of interest as starting material for the synthesis of the nitrogen-containing heterocyclic compounds. Pinocarvone oxime 1, being simple and easily available derivative of α-pinene, was used for the preparation of isoxazole, pyrazole, pyrazoline, and indole heterocycles.



The oxidative reactions of oxime 1 with I_2-$NaHCO_3$ [1] and $NaNO_2$-AcOH produce isoxazole 2 and nitropyrazole 4 correspondingly. The first compound is used for the synthesis of pyrazole 3, which is obtained by treatment with $N_2H_4 \bullet H_2O$ in a sealed tube. Hydrazinolysis of the starting oxime in acetic acid results in the formation of acetylpyrazoline 5. For the preparing of compound 7 we have developed synthetic pathway via intermediate N-nitroimine. The pinocarvone oxime 1 is reduced with N_2H_4 to the isopinocamphone oxime, which is oxidized by a known procedure [2] to N-nitroimine 6. The last one is transformed to the indolenine 7 by two-stage process involving the treatment with phenylhydrazine and the heating.

The nitrogen-containing heterocycles are regarded as potential biologically active substances and the excellent ligands for the coordination chemistry. The optically active pinane heterocycles are prospective chiral auxiliary for enantioselective synthesis [3].

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STRUCTURE REVISION OF THE LICHEN PIGMENT HAEMOVENTOSIN

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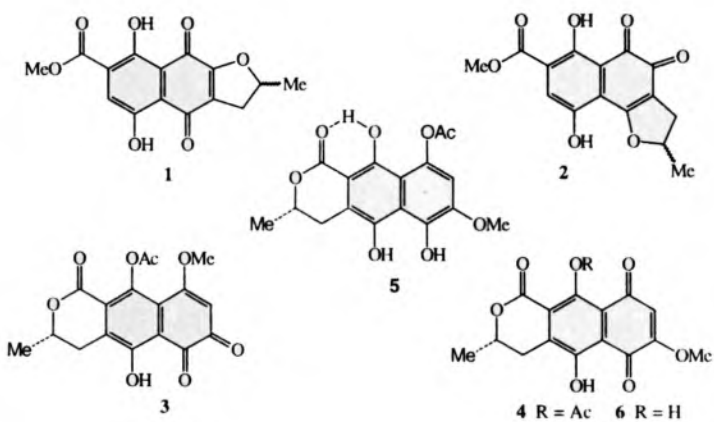
and
Siegfried Huneck and Uwe Himmelreich,
Institute of Plant Biochemistry, Halle(Saale), Germany.

Haemoventosin, a deep red pigment produced by the lichens *Ophioparma ventosa* (syn. *Haematomma ventosum*) and *O. lapponica*, presented a structural problem which at first sight appeared to be ideal for the application of nmr experiments producing connectivity information, but ultimately it was a chemical reaction (applied in the nmr tube) that solved the problem of distinguishing *ortho*- and *para*- quinonoid isomers.

Bruun and Lamvik proposed structure **1** in 1971, mainly on the basis of ^{1}H nmr spectroscopy and the loss of the methoxyl group on saponification; more recently reaction with *o*-phenylene diamine was reported (Maksinov *et al.*, 1990) to form a derivative assumed to be a quinoxaline, leading to the proposal of structure **2**, containing an *ortho*- rather than a *para*-quinone.

Connectivity experiments with the mono-acetate of haemoventosin led to the alternative structures **3** and **4**. Reduction gave a product **5** containing three OH groups, one of which resonated notably at δ_H 12.53, indicative of strong hydrogen-bonding to the lactone carbonyl group. Acetate migration to produce **5** is only possible following reduction of the *para*-quinonoid **4**, and indicates that the structure of haemoventosin is **6**.



NEW CONSTITUENTS OF *LACTARIUS VELLEREUS*

Włodzimierz M. DANIEWSKI, Dorota PRZESMYCKA, Maria GUMUŁKA, Waldemar ANCZEWSKI

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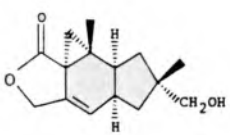
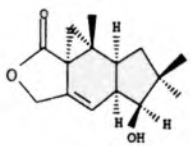
Reinvestigation of monohydroxylactone fraction of ethanolic extract of *Lactarius vellereus* afforded four new monohydroxylactones possessing marasmane skeleton and a new dihydroxy-furan with lactarane skeleton. Structures of compounds **1,2,3,4** and **5** were established by extensive spectroscopic investigations. Compound **2** when reacted with trichloroacetic isocyanate gave a crystalline derivative which was investigated by X-ray, confirming its structure.



1.



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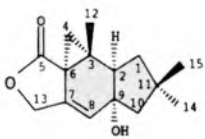

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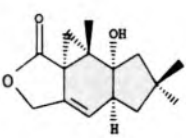

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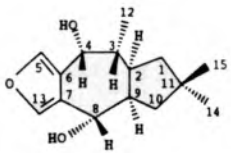

5.

Spectroscopic data as well as biogenetic problems concerning these compounds will be presented.

BIOLOGICALLY ACTIVE SESQUITERPENE LACTONES GAILLARDIA GRANDIFLORA HORT.

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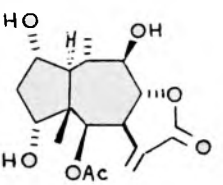
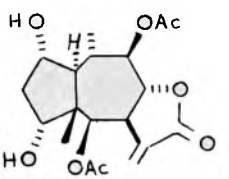
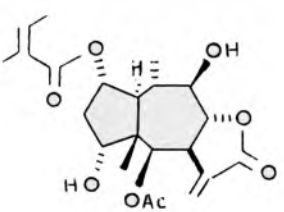
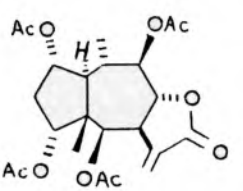
From the flower baskets and leaves of *Gaillardia grandiflora Hort*, there have been isolated three sesquiterpene lactones, two of which proved to be new ones and were named gaigranin (I) and gaigrandin (II), third was identified as pseudoguaianolide spatulin (III).

Extraction by chloroform, acetone and water with the following chromatography on the column with silicagel proved the quantitative yield of lactones from the aerial part of this plant with the following treatment of the isolated amount of substances by mixture of ethanol and water (2:1).

Gaigranin (I) is a colourless crystalline substance of composition $C_{17}H_{27}O_7$, m.p. 186-189°C (from ethanol), $[\alpha]_D^{20}$ +17,3 (c, 0,52, ethanol).

X-ray structural study of the spatulin (III) molecule , comparing of its NMR spectra with those of gaigranin (I) and of the products of their complete O-acetylation, that turned out to be identical compounds (IV), and, at last, analysis of two-measured spectrum NMR (I) allow to discuss this lactone as 2(S),4(R),9(R)-trihydroxy-6(R)-acetoxy-1(S),7(S),8(R),10(R)-pseudoguai-11(13)-en-8,12-olide.

Gaigrandin (II) is a colourless crystalline substance of composition $C_{22}H_{30}O_8$, m.p. 205-208°C (from ethanol). Structure of gaigrandin (II) and conformations of its cycles have been studied by method of X-ray structural analysis. Here at, it has been determined that its lactone cycle has a conformation C-7 of convert and the seven-membered cycle has a shape

which is interstitial between the conformations chair and twist-chair. The five-membered cycle A in molecule (III) is in the form of C-5 convert. Obtained data also indicate at presence of two hydroxy and one acetoxygroups and presence of angelic acid remains at C-2. So, gaigrandin has a structure of 2α -angeloiloxy-4α,9βdihydroxy- 6β-acetoxy-7α,8β , 10α(H)-pseudoguai-11(13)-en-8,12- olide(II).

Repellent, anti-feedant and anti-tumour activities of gaigranin (I) have been determined.

The research described in this publication was made possible in part by Grant № MYN000 from the International Science Foundation.

ALUMINACYCLOPENTANES IN A SYNTHESIS OF ISOPRENOID STRUCTURE HYDROCARBONS

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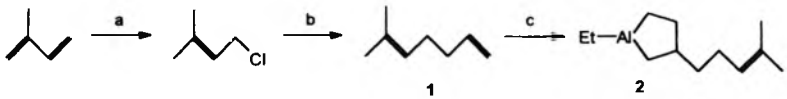
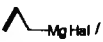
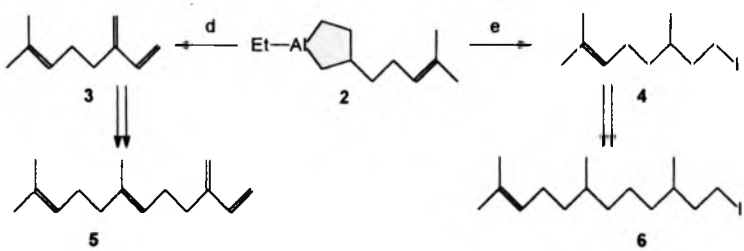
This paper describes results of studies on aluminacyclopentanes [1] used in a regioselective synthesis of isoprenoids in the presence of homogeneous metallacomplex catalysts. A synthesis was based on a cyclometallation of isoprene and its derivatives with alkylalanes. Thus, cycloalumination of 2-methyl-hepta-2,6-diene **(1)** with $AlEt_3$ effected by a catalytic amount of Cp_2ZrCl_2 under mild conditions leads to 2-alkenyl-aluminacyclopentane **(2)** of high regioselectivity.



a : HCl; b : 

; c : $AlEt_3$ / [Zr]

Aluminacyclopentane **2** transforms to myrcene **(3)** in 70% yield without preliminary elimination in the presence of equimolar amounts of allylacetate and freshly distilled $Ni(acac)_2$ (5 mol%) in ether. A reaction of alumina-cyclopentane **2** and 1,2-diiodoethane in THF leads to dimethyliodooctene **(4)** in 90% yield. A futher increase of a chain by analogous transformations gives pharnezene **(5)** and iodoolefin **(6)** of isoprenoid structure according to the following Scheme :



d : 

; e : 

The synthetic routes proposed give novel possibilities in a synthesis of regular E-isoprenoids.

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NEO-CLERODANE AND LANGUIDULANE DITERPENOIDS FROM *Salvia ramosa* AND *S. karwinskii* (LABIATAE)

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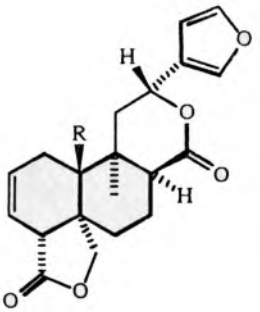
Instituto de Química de la Universidad Nacional Autónoma de México. Circuito Exterior, Ciudad Universitaria, Coyoacan 04510, México D.F.

In a continuation of our systematic studies of Mexican *Salvia* species[1] we have analysed the diterpenoid content of *Salvia ramosa* Brandegee (*Salvia* Section Scorodonia) and *Salvia karwinskii* Benth (*Salvia* Section Holwaya).

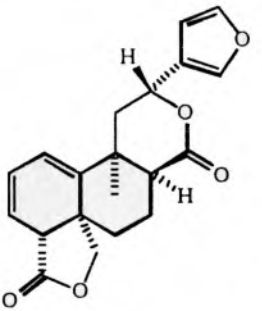
The aerial parts of *Salvia ramosa* afforded, after extensive chromatographic purification, two neo-clerodane diterpenoids, related to salviarin (**1**), identified as 10β-hydroxysalviarin (**2**) and 1(10)-dehydrosalviarin (**3**), previously isolated from *Salvia herbacea* and *S. lineata* [2] respectively. Compound **3** showed a potent antifeedant activity against larvae of *Spodoptera littoralis* [3] and for adult western corn rootworm *Diabrotica virgifera virgifera* LeConte, with a ED_{50} of 1.1 nmol/disk. In this bioassay compound **3** can approach azadirachtin (ED_{50} of 0.39 nmol/disk) in antifeedant potency[4].

In addition a new languidulane[5] type diterpenoid (**4**) was isolated as a crystalline solid whose structure was determined by spectroscopic means. Compound **4** ($C_{22}H_{24}O_7$ MS) showed in its ^1H NMR spectrum two one-proton doublets (J = 2Hz) at δ 7.3 and 6.7 characteristics of the C-14 and C-15 protons of a languidulane diterpenoid with an α,β substituted furan ring[5]. A double doublets at δ 7.07 was ascribed to H-3. An AB system observed at δ 4.96 and 4.12 (J = 9 Hz) was assigned to the C-19 methylene protons. The chemical shift of this system indicate the presence of an acetate group at the C-7 α axial position. A double doublet at δ 5.15 was assigned to the geminal proton of this acetate group and a broad doublet at δ 4.02 was ascribed to the geminal proton of a hydroxy group located at C-6 position. The orientation for this hydroxy group was established with the aid of the coupling constant (J = 2.2) observed for H-6. An AB system at δ 2.96 and 2.63 (J = 14.5) was ascribed to the C-11 methylene protons. Another languidulane-type diterpenoids have been isolated previously from *Salvia languidula* , *S. sousae* and *S. urolepis* [6].

From the aerial parts of *Salvia karwinskii* only small amounts of compound **3** was isolated. The presence of this diterpenoid in *S. karwinskii* has chemotaxonomic interest since *Salvia lineata*, from which 1(10)-dehydrosalviarine (**3**) was first isolated[2] is included in Section Fulgentes, a section botanically related to Sect. Holwaya.



1. R = H
2. R = OH



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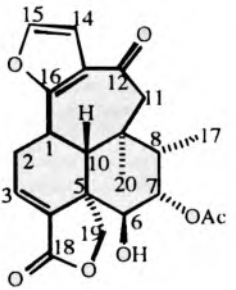

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4. Prof. Christopher Mullin, Department of Entomology Pennsylvania State University USA. Personal Communication.
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SCUTERULEINES A-C .NEW NEO-CLERODANE DITERPENOIDS FROM *Scutellaria caerulea* (LABIATAE).

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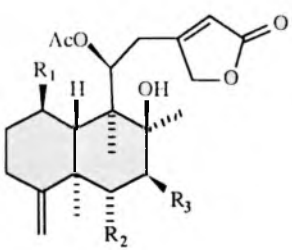
Circuito Exterior, Ciudad Universitaria, Coyoacan 04510, México D.F.

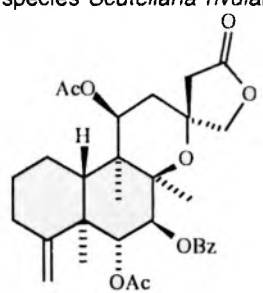
Scutellaria L. is a large subcosmopolitan genus of the Labiatae with ca 360 currently recognized species.Recently, plants belonging to this genus have attracted much attention owing to interesting biological activities found for some neo-clerodane diterpenoids isolated from them (1). In México, *Scutellaria* is represented by ca 36 species, most of them growing in the mountains near the centre of the country. As a part of our ongoing search for diterpenoids from plants of the Labiatae, with potential antifeedant activity, we have started to study Mexican *Scutellaria* species. Recently we describe the structure of three new neo-clerodane diterpenoids from *Scutellaria drummondii* Benth (2).

In this report we describe the structure of three new neo-clerodane diterpenoids, named scuteruleines A-C (**1-3**), isolated from *Scutellaria caerulea* Moc. et Sesse. The structure of compounds 1-3 were established from their spectroscopic data including extensive NMR analysis (COSY, HETCOR, HMBC and HMQC).

From a chemotaxomomic point of view, it is of interest to note that **1-3** lack an oxygenated substituent at C-19 and hence the hemiketalic 19-2α function, commonly found in European *Scutellaria spp*. The diterpenoids of *Scutellaria caerulea* share these features with the diterpenoids of *S. drummondii* (2) and with some neo-clerodane diterpenoids isolated from the Chinese species *Scutellaria rivularis* (3).



1. R_1 = OH, R_2 = R_3 = OCOCH(CH$_3$)$_2$
2. R_1 = R_2 = OAc, R_3 = OBz



3.

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3. Lin Y.L., Kuo Y.H. *Chem Pharm. Bull*. **37, 583 (1989)** and references cited therein..

SEX PHEROMONE OF PINE SAWFLIES. SYNTHESIS OF A BIOLOGICALLY ACTIVE COMPONENT ISOLATED FROM THE PINE SAWFLY *MICRODIPRION PALLIPES*.

Erik HEDENSTRÖM,[*] Ann-Britt WASSGREN,[**] Olle ANDERBRANT,[***] Gunnar BERGSTRÖM,[**] and Hans-Erik HÖGBERG[*]

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[**] Department of Chemical Ecology, Göteborg University, S-413 19 Göteborg, Sweden

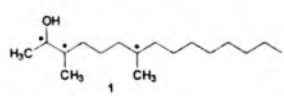
[***] Department of Ecology, Lund University, S-223 62 Lund, Sweden

Several pine sawflies use esters of one ore two stereoisomers of 3,7-dimethyl-pentadecan-2-ol **1** (diprionol) as sex pheromone, among them *Neodiprion sertifer*.[1]

Previously we have found that the pine sawfly *Diprion pini*, uses one stereoisomer of a propionylated or acetylated homologue to diprionol, namely (2*S*,3*R*,7*R*)-3,7-dimethyltridecan-2-ol **2** as male attractant.[2]

Recently we have found that yet another pine sawfly, *Microdiprion pallipes*, uses a non diprionol-based sexual pheromone namely, 3,7,11-trimethyltridecan-2-yl propionate **3**. Most probably one of the four isomers of this propionate with S-configurations at carbons 2 and 3 is the biologically active stereoisomer.[3] Results from GC-EAD measurements and field tests will be presented.

Synthesis from geraniol and 3,4-dimethyl-(γ)-butyrolactone of stereoisomeric mixtures containing the biologically active compound will be described. The syntheses of the four isomers mentioned above in stereoisomerically pure forms are under way.



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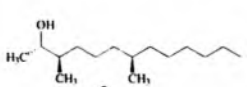

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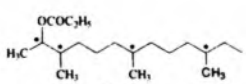

3

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REGULATION OF ISOPENTENYL DIPHOSPHATE ISOMERASE IN ELICITOR-TREATED *CINCHONA ROBUSTA* CELL CULTURES

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Elicitation of *Cinchona robusta* cell suspension cultures with a *Phythophthora cinnamomi* preparation resulted in a *de novo* synthesis and accumulation of Rubia-type anthraquinones (AQs). The biosynthesis of AQ phytoalexins is preceded by a transient induction of isopentenyl pyrophosphate (IPP) isomerase activity and a transient inhibition of farnesyl diphosphate synthase (FPS). IPP isomerase catalyzes the interconversion of IPP and dimethylallyl pyrophosphate (DMAPP), both essential intermediates in the terpenoid biosynthesis. Alternatively, DMAPP can be diverted to AQ biosynthesis. From higher plants and cell cultures no studies have been reported on the role of IPP isomerase in the anthraquinone pathway.

The elicitor-inducible IPP isomerase was purified to homogeneity in four chromatographic steps. Two isoforms of IPP isomerase with different sizes (30 and 34 kDa) and small differences in their affinity for IPP (both K_m < 10 µM) and cofactor requirements (one of both Mn^{2+} and Mg^{2+}) were present in elicited cell cultures. Geranyl diphosphate, an intermediate in the terpenoid biosynthesis, inhibited the enzyme in a competitive manner with a K_i of about 110 µM. Immunoblot analysis using anti-IPP isomerase antibodies reveled also the presence of these two isoforms in the elicited *C. robusta* cell protein extract.

Our results suggest that the induced biosynthesis of anthraquinones in the *C. robusta* cells comprise complex regulation to satisfy the IPP and DMAPP demands: induction of IPP isomerase activity via a transient, but strong, induction of one of the IPP isomerase isoforms, and inhibition of the FPS activity, which leaves more substrate (IPP) for the IPP isomerase and which reduces the feedback inhibition by prenyldiphosphates.

INTRAMOLECULAR ASSOCIATION OF THE 17α-HYDROXY GROUP IN E-HOMOLUPANE DERIVATIVES

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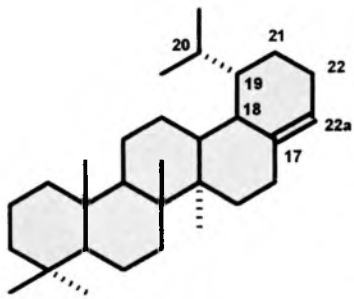
A series of derivatives with various oxygen functionalities in positions 17,22a or 19,20 was prepared by addition and oxydation reactions from E-homolupane derivatives I and II. The structures of the prepared compounds were confirmed by ^{1}H NMR, ^{13}C NMR and infrared spectroscopy.



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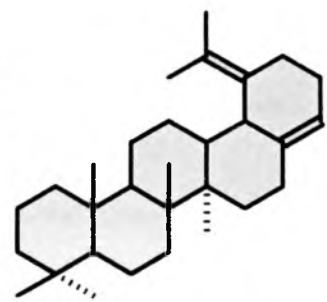

II

In connection with modification of the side chain and substitution in position 22a, the kind of intramolecular association of 17α-hydroxy group, which can form hydrogen bond with an acceptor in the side chain as well as with neighbouring group in position 22a, was then studied. The following conclusions can be made from infrared measurements of intramolecular hydrogen bonds.

1. In 17,22a disubstituted derivatives with the isopropyl group in position 19 the 17α-hydroxy group forms an intramolecular hydrogen bond with the neighbouring group.
2. When the α-epoxy group is present in position 19,20 then, because of its basicity and good steric accesibility, the 17α-hydroxy group forms a hydrogen bond only to the epoxy group, irrespective of substituent in neighbouring position 22a.
3. 17α-Hydroxy derivatives with an oxygen functionality in position 22a, containing the 19(20) double bond, exist in the tetrachloromethane solution as an equilibrium mixture of two forms with intramolecular hydrogen bond involving either the double bond or the neighbouring 22a substituent, the latter beeing weaker (smaller $\Delta\nu_{OH}$).
4. The 19-oxo group does not participate in the association and thus the 17α-hydroxy group forms hydrogen bond only with substituent in position 22a.

5. 22aα-Hydroxy derivatives exhibited only a free hydroxyl band, irrespective of the type of the side chain because the distance of the hydroxy group from the potential acceptor is too great.
6. Judging from the weak hydrogen bond and mutual orientation of the 17α-hydroxy group and 22a-keto group in prepared ketols, we assume their association by means of π-orbitals of the keto group[1]. In 22aα-monoacetates the 17α-hydroxyl associates with the free electron pair of the alkoxy group[2].

The authors are grateful for the financial support from the Grant Agency of the Czech Republic (Reg. n. 203/93/2468).

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AUTOXIDATION OF UNSATURATED DIALDEHYDES GENERATES BIOACTIVE PRODUCTS

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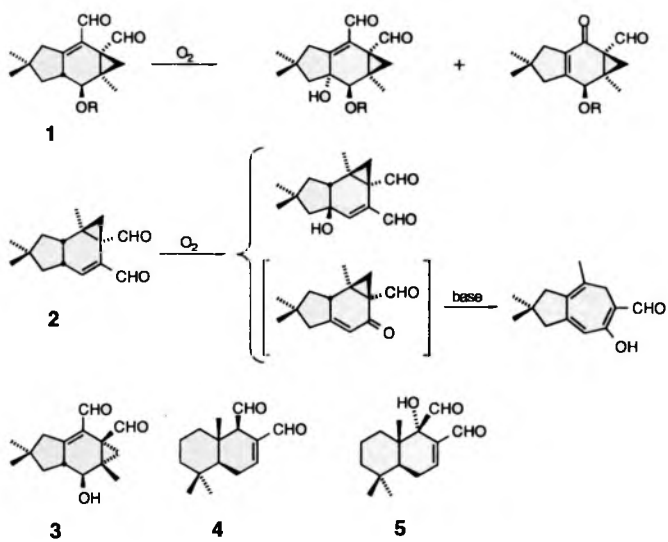
Sesquiterpenoids containing an unsaturated dialdehyde functionality and possessing, for example, potent antibiotic and antifeedant activities, have been isolated from various organisms such as plants, fungi, molluscs, etc., and several studies have demonstrated the necessity of both aldehyde groups as well as the double bond for their biological activities.[1,2]

We propose that bioactive products formed by autoxidation of the two mutagenic and anti microbial sesquiterpenes merulidial (**1a**, R=H) and isovelleral (**2**) in bioassay media are partially responsible for the activities of the two compounds.[3] The biological activities of the autoxidation products are, as far as has been possible to assay, of the same order as the parent compounds, and it is shown that they are formed in normal bioassay media. Merulidial (**1a**) is especially interesting, as its rate of autoxidation is considerably higher compared with the non-mutagenic merulidial derivatives **1b** (R=Ac), **1c** (R=TBDMS) and **3**. Similar transformations of the non-mutagenic sesquiterpene polygodial (**4**) and the mutagenic sesquiterpene warburganal (**5**) will also be discussed.



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TRITERPENOIDS IN *ILEX PARAGUARIENSIS* CELL CULTURES

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Ilex paraguariensis St. Hil. (Aquifoliaceae) is a brazilian tree from which leaves and fine branches are used by people from south of Brazil, Argentina, Paraguay and Uruguay to prepare a traditional beverage called 'mate'. Its leaves are also used in popular medicines as a diuretic, an antiinflammatory, an antirheumatic, stimulant of the central nervous system and inhibitor of the appetite.

Phytochemical investigations on the leaves of this species indicated the presence of many triterpenoids as α-amyrin and ursolic acid (Mendive, 1940) and saponins (Gosmann et al., 1989, 1995).

For studying the biosynthesis of these triterpenoids cell cultures have been iniciated. First studies of the triterpenoids contents of these cells showed the presence of such compounds. Both qualitative and quantitative differences were observed for the same cell line grown into two different laboratories. Also clear differences were observed if compared with leaf extracts.

No xanthine derivatives (caffeine, theobromine, theophylline) could be detected in the cell cultures.

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Molluscicidal natural products.

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Abstract not received

REDUCTIONAL REARRANGEMENT OF α- SANTONIN

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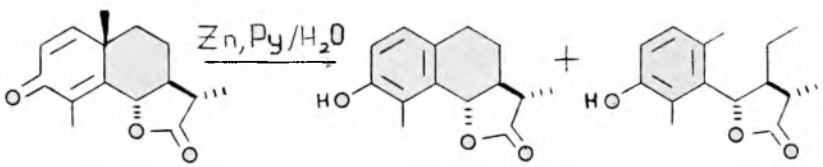
Pecularities of the structure of sesquiterpene lactone of α -santonin which is common for *Artemisia* species make it possible to discuss it as a model object for skeleton rearrangement[1,2]. We performed the reductional rearrangement of given eudesmanolide in boiling mixture of pyridine/water (15ml/1ml) using the zinc dust activated by 5% hydrochloric acid. As a result of the reaction, there were formed the phenol derivatives of terpenoids (2) and (3) giving characteristic yellow colouring at effecting of nitrogen dioxide to the TLC plates. The structures of (2) and (3) were determined on the base of IR and NMR spectral data.



(1) (2) (3)

In NMR spectra of (2) and (3) there are present the signals characteristic for methyl groups of aromatic ring (singlets at 2.21, 2.28 and 2.34 ppm.),doublets of protons H-1 and H-2 (6.67 ppm.,J=8Hz(1H); 6.70 ppm., J=8Hz(1H); 6.64 ppm., J=8Hz(2H)), doublets of lactone protones H-6 (5.43 ppm., J=9Hz (1H); 5.01 ppm.,J=9Hz(1H)), doublets of methyl groups H-13 (1.37 ppm., J=7Hz(3H); 1.27 ppm., J=7Hz(3H)), and also the characteristic triplet of methyl group in product (3) in region of 0.88 ppm., J=7Hz which correspondes to protones H-9.

Produced phenolderivatives (2) and (3) showed high antimicrobal, antifungal and antioxidation activities.

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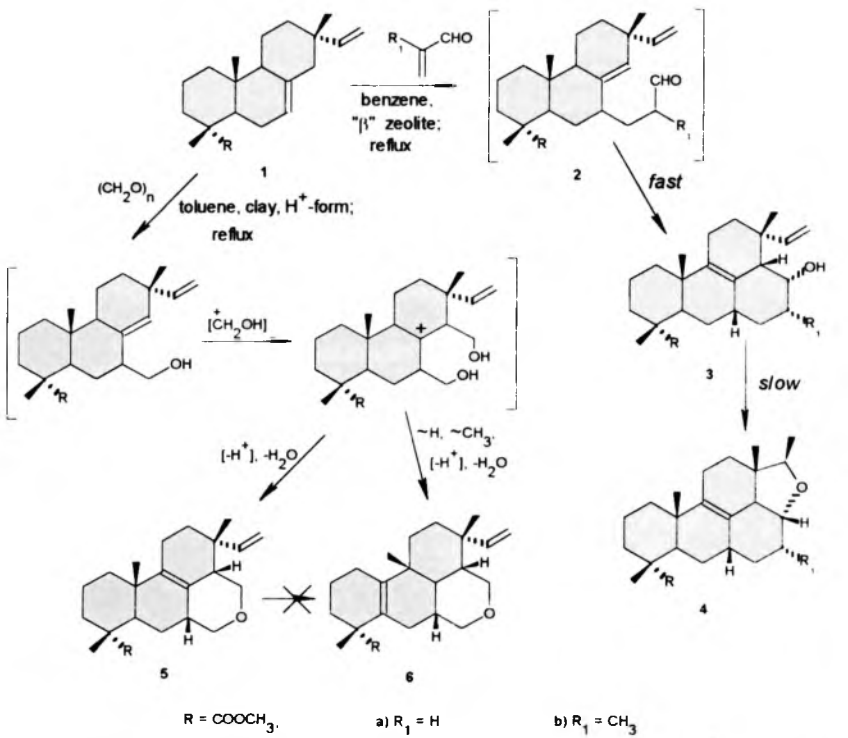
The research described in this publication was made possible in part by Grant № MYN000 from the International Science Foundation.

CYCLOADDITION OF SIMPLE CARBONYL COMPOUNDS TO METHYL ISOPIMARATE USING ALUMOSILICATE CATALYSTS.

Eugeny V. KUZAKOV, Emma N. SHMIDT, Dina V. KORCHAGINA, Irina Yu. BAGRYANSKAYA, Tatjana V. RYBALOVA, Yurii V. GATILOV, Kazimira G. IONE, Vladimir A. BARKHASH
Novosibirsk Institute of Organic Chemistry, Acad. Lavrentjev Ave. 9, Novosibirsk 630090, Russia

Methyl isopimarate (1), diterpene olefine having isolated double bond, comparatively easily reacts with simple carbonyl compounds in alumosilicate's presence. Its interactions with acroleine or α-methylacroleine proceed mainly by $\Delta^{8,14}$-ene condensation pathway. Products of this condensation (2a) and (2b) are shown to cyclize under given conditions by intramolecular ene-like mechanism to result in alcohols (3a) or (3b). The last products, in turn, may partially convert to diesters (4a) and (4b) correspondingly with cyclic system of new type.



Interaction of compound (1) and paraform in presence of "ascanite-benthonite" clay takes place as an addition of two CH_2O equivalents; further dehydratation of intermediate formed leads to tetracyclic tetrahydropyrane-type products (5) and (6).

All structures (3)-(6) were confirmed by ^1H and ^{13}C NMR data, including LRJMD and 2D-INADEQUATE methodics, and also by X-Ray analysis.

SESQUITERPENE BIOSYNTHESIS IN HAIRY ROOT CULTURE OF *LACTUCA VIROSA*: INFLUENCE OF N,N-DIMETHYLDODECYLAMINE N-OXIDE

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Institute of Pharmacology, Department of Phytochemistry,
Polish Academy of Sciences , 12 Smętna Street, 31-343 Kraków, Poland

Hairy root culture of Lactuca virosa was derived from "in vitro" grown, aseptic plantlets infected with Agrobacterium rhizogenes strain LBA 9402. The hairy roots were cultivated in MS medium with macronutrients reduced to a half (MS 1/2 macro) on a gyrotory shaker (110 r.p.m.) in the dark. Genetic transformation was proved by rifampicin resistance of the tissue, as well as by opine biosynthesis.

NND (N,N-dimethyldodecylamine N-oxide) causes inhibition of cyclization of 2,3-oxidosqualene to cycloartenol. To investigate the effect of NND content on the biomass accumulation and sesquiterpene lactone biosynthesis, a series of liquid MS 1/2 macro media containing four different concentrations of 0.2 % NND in MeOH (250 mg/l, 500 mg/l, 750 mg/l and 1000 mg/l) were prepared. The hairy roots were harvested after three weeks of growing in the above mentioned media and were analysed by HPLC.

Growth indexes (GI) increased proportionally to the NND concentration employed (up to 750 mg/l) and then decreased. The best GI (5.08) was in the medium containing 750 mg/l of 0.2 % NND.

RP-HPLC analyses of the examined plant material (μ Bondapak C18 column, 2mm x 30cm, MeOH-H_2O, 35:65, isocratic mode, flow rate 0.5 ml/min) showed quantitative (but not qualitative) differences in sesquiterpene lactone contents in comparison to the control.

NEW CHIRAL CONDENSING REAGENTS. SYNTHESIS AND APPLICATION OF TERPENOXY-s-TRIAZINES FROM CYANURIC CHLORIDE AND ALCOHOLS WITH PINANE AND OTHER TERPENIC SKELETON.

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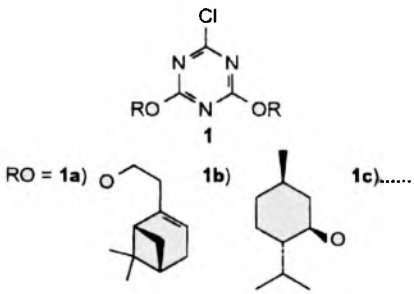
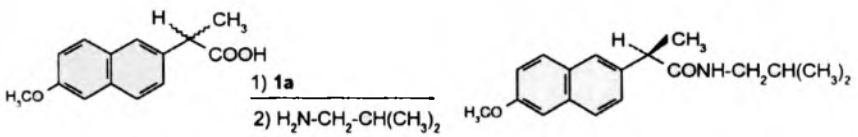
Institute of Org. Chemistry, Technical University of Łódź, ul. Żwirki 36, 90-924 Łódź, POLAND

The terpenic alcohols derived mainly from pinenes were used for synthesis of chiral triazines **1,** which act as highly efficient condensing reagents[1].



Terpene residue was used as a good chiral auxiliary[2] for enantioselective activation of carboxylic acids and enantioselective acylation of amines, amino acids and peptides.



Supported by the State Committee for Scientific Research (KBN), Grant 3 T09A 067 08 and Technical University of Łódź, Grant I-18/18/95/Dz.S.

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STUDIES ON RETINAL PROTEINS

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Department of Chemistry, Columbia University, New York, NY 10027, U.S.A.

A general acount will be given on past and ongoing studies on retinal proteins, mostly on the visual pigment rhodopsin. The topics to be discussed include the following.

1) Why Nature has chosen 11-cis-retinal as the common visual chromophore.
2) The triggering mechanism of visual transduction.
3) The location of the retinal chromophore within the binding cavity and the visual transduction process as studied by photoaffinity labeling.
4) The current status of bleaching adaptation, a phenomenon less understood than vision.
5) Solid state NMR studies of bacteriorhodopsin regarding the direction of the methyl groups in the retinal side-chain (if time permits).

1,6-GERMACRADIEN-5-OL A MAJOR COMPONENT OF THE DEFENSE SECRETION OF THE PINE SAWFLY *NEODIPRION SERTIFER* LARVAE

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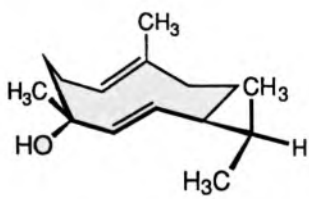
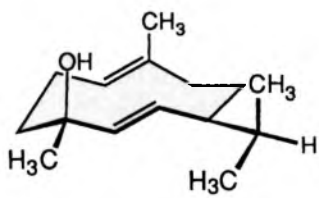
The pine sawfly (*Neodiprion Sertifer*) is a pest on Scots Pine, *Pinus sylvestris*, and other *Pinus* species in the northern parts of Europe. The larvae feed on the pine needles and a severe attack causes a considerable disturbance in the growing process of the tree. The larvae has an interesting defense mechanism towards its enemies (mainly ants). When attacked, the larvae discharge viscous droplets causing its enemy to retreat[1]. Earlier workers[1,2] have shown that the larval discharge mainly consists of resin acids and monoterpenes. When Bergström et al[3] reinvestigated these droplets they identified an additional major component from the neutral parts of the extract. They found that it was identical with 1,6-germacradien-5-ol **1**, which is the major sesquiterpene in the extract. They also found that it was present in the extract of pine needles on which the larvae feed albeit in much lower concentrations.

The interesting biological activity of these droplets could, at least in part, be due to 1,6-germacradien-5-ol. In order to supply this compound for studies of its biological activity we are presently studying possible synthetic approaches to **1**. Therefore knowledge of the absolute configuration is essential. To establish this, we have isolated the pure 1,6-germacradien-5-ol from the needles of *Pinus sylvestris*. Isomerization of 1,6-germacradien-5-ol **1** in formic acid[4] to (-)-α-cadinol revealed the configuration of the isopropyl group. This left us with the two possible diastereomers **5S,8S-1** and **5R,8S-1**. We are presently investigating the absolute configuration further by chemical degradation and hope to obtain a crystalline derivative suitable for X-ray crystallography.

The 10-membered ring of 1,6-germacradien-5-ol can adopt several conformations. MM2 calculations suggests that the two diastereomers mentioned above should have different conformations. **5S,8S-1** should prefer a "chair-chair" conformation while **5R,8S-1** should prefer a "boat-chair" conformation. We have used NOESY and VT-NMR measurements in order to correlate the spectral data with the results of the MM2 calculations.



5R,8S-1



5S,8S-1

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RACEMIC α-TOCOPHEROL, (E,E)- AND (Z,Z)-α-TOCOTRIENOLS AND THEIR ISOPRENOLOGS BASED ON A PARTIAL OZONOLYSIS OF ISOPRENE OLIGOMERS AND POLYMERS

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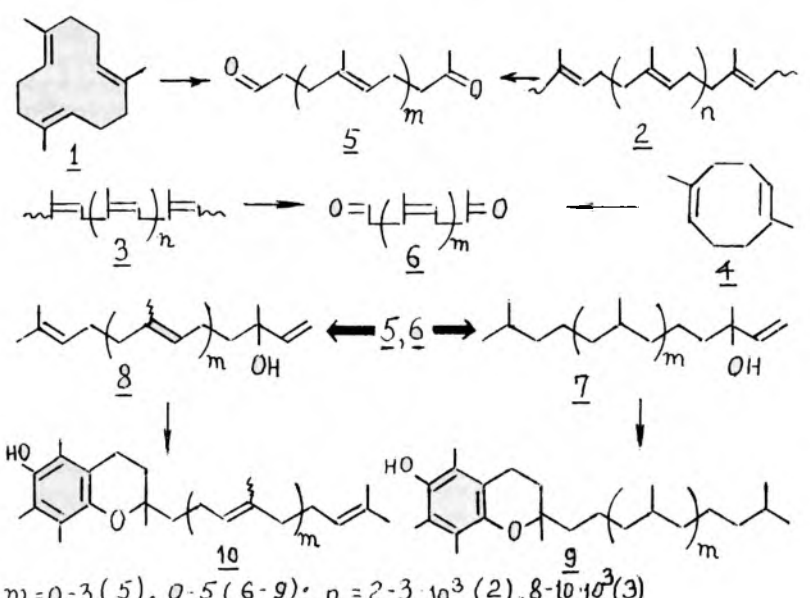
A partial ozonolysis of regular isoprene cyclotrimer (**1**) by a calculated amount of ozone leads mainly to sesquiterpenoid ketonic aldehyde **5** (m=2) of (E,E)-configuration - a product of a splitting of one multiple bond in **1**. Terpenoids **5** (m=1 and 0) were formed as by-products (10-20%). In a partial ozonolysis of (E)-1,4-polyisoprene(**2**)with a further rectification of isoprenologs the ketonic aldehydes **5** were isolated with a number of (E)-isoprene units up to 3, whereas individual 1,4-polyene isoprenoid ketonic aldehydes **6** of (Z)-series were isolated with a number of isoprene units (m) to 5 from a product of ozonolysis of (Z)-1,4-polyisoprene (**3**). The first two of them (m=0 and 1) were formed also by ozonolysis of 1,5-dimethyl-1,5-cyclooctadiene (**4**). Isophytol (**7**, m=2) and its triene analogs (**8**, m=2) of (E)- and (Z)-series, and their isoprenologs were synthesized by successive transformations of isoprenoids ketonic aldehydes **5** and **6**. Target α-tocopherol (**9**, m=2), (E,E)- and (Z,Z)-α-tocotrienols (**10**, m=2) and their isoprenologs were synthesized by a condensation of each of them with 2,3,6-trimethylhydroquinone.

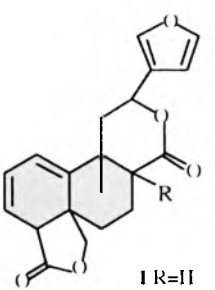


CLERODANIC DITERPENES FROM *SALVIA HERBACEA*

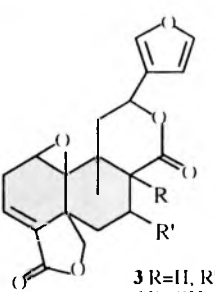
Alfredo ORTEGA
Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México.

A large number of species of the Labiatae family so far analyzed, contain diterpenes of clerodanic and abietanic skeletons and also triterpenes with different types of structures are common[1,2].

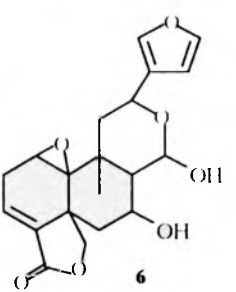
Recently, the search for pharmaceutical useful substances was conducted almost systematically within an order or family of plants. This is the case of the *Salvia* genus, since many species of this genus are used in folk medicine. Diterpenes, phenolic and volatile compounds isolated from this genus are responsible of their biological activity[3]. In our search for new molecules, we investigated the aerial parts of *Salvia herbacea*. Multiple column chromatographies of the acetone extract, allowed to isolate a series of substances chemically related. One of them (**1**) is a known compound[4] and the other five (**2-6**) are novel molecules. Extensive NMR spectroscopy (1D and 2D techniques as DEPT, COSY, normal and long-range heterocosy, etc) were used for structure elucidation of the new natural products.



1 R=H
2 R=OH



3 R=H, R'=OH
4 R=OH, R"=H
5 R=R'=H



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LANGUIDULANE AND 5,10-SECOCLERODANE DITERPENES FROM *SALVIA TONALENSIS*

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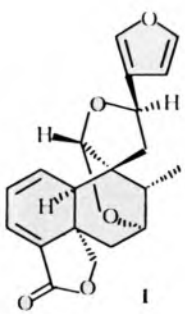
As a part of our chemical studies on Mexican *Salvia* species, we have investigated the constituents of *Salvia tonalensis* Brand.

The acetonic extract of the aerial parts of this species afforded, after extensive column chromatography, the known diterpenes salvifaricine (**1**) and salvisousolide (**2**). In addition, two new compounds were isolated, the languidulane tonalenine (**3**) and the 5,10-secoclerodane, tonalensine (**4**). Structure of compound **3** was established by spectral means, while that of tonalensine, which exist as a mixture of conformers in solution, was determined by X- ray analysis.



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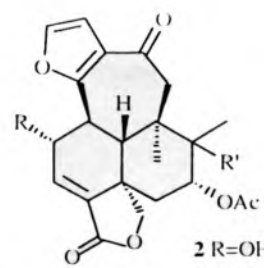
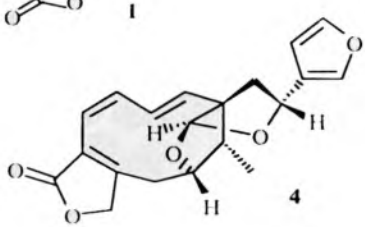


2 R=OH, R'=βH
3 R=H, R'=αOH

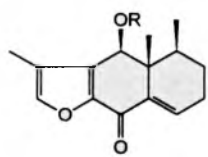


4

SESQUITERPENES AND RELATED COMPOUNDS FROM *ADENOSTYLES ALPINA* WITH EFFECT ON INSECT HERBIVORES.

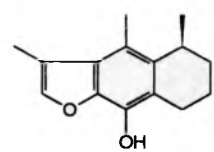
Milan PAVLÍK, Juraj HARMATHA, Miloš BUDĚŠÍNSKÝ, David ŠAMAN, Jitka KOHOUTOVÁ and Věra LAUDOVÁ
Institute of Organic Chemistry and Biochemistry AV ČR, 166 10 Prague, Czech Republic

Sesquiterpenes of eremophilane type are characteristic constituents of plant species belonging to *Senecioneae* tribus of the *Asteraceae* family. Some of these compounds are known as effective insect antifeedants. It was interesting to learn what influence they have on leaf beetles, insect herbivore specialists, naturally feeding on *Senecioneae* plants. The composition of sesquiterpenes in these plants is prevailably known with exception of *Adenostyles alpina* (L.) Bluff & Fingerh.; syn.: *A. glabra* (Miller) DC., an ecologically significant host plant for leaf beetles. From the rhisomes of *A. alpina* adenostylone (**1**) and neoadenostylone (**2**) were isolated and identified. Both compounds were described long ago as constituents of *A. alliariae*. Cacalol (**3**), a biogenetically related compound frequently occurring in several *Senecioneae* plants, has been also found in *A. alpina* as well as in *A. alliariae*. From *A. alpina* a new trimeric compound **4** based on cacalol has been isolated and identified. A low content of adenostylone and neoadenostylone in leaves excluded these two compounds as significant ecological components. However, cacalol and cacalol-trimer are further investigated as compounds influencing the variability of leaf beetles population on *Adenostyles* species.

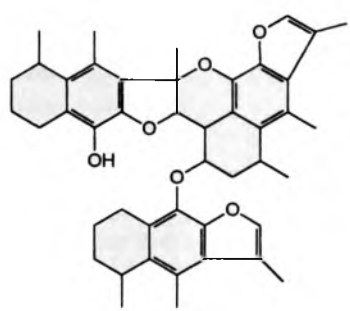


1. R = $COCH(CH_3)_2$ adenostylone

2. R = $COCCH_3{=}CHCH_3$ (*trans*) neoadenostylone



3. cacalol



4. cacalol - trimer

MOLECULAR REARRANGEMENTS OF CIS- AND TRANS-2,3-EPOXY CARANES IN SUPERACID

Marina P.POLOVINKA, Oleg G.VYGLAZOV, Dina V.KORCHAGINA, Vladimir A. BARKHASH
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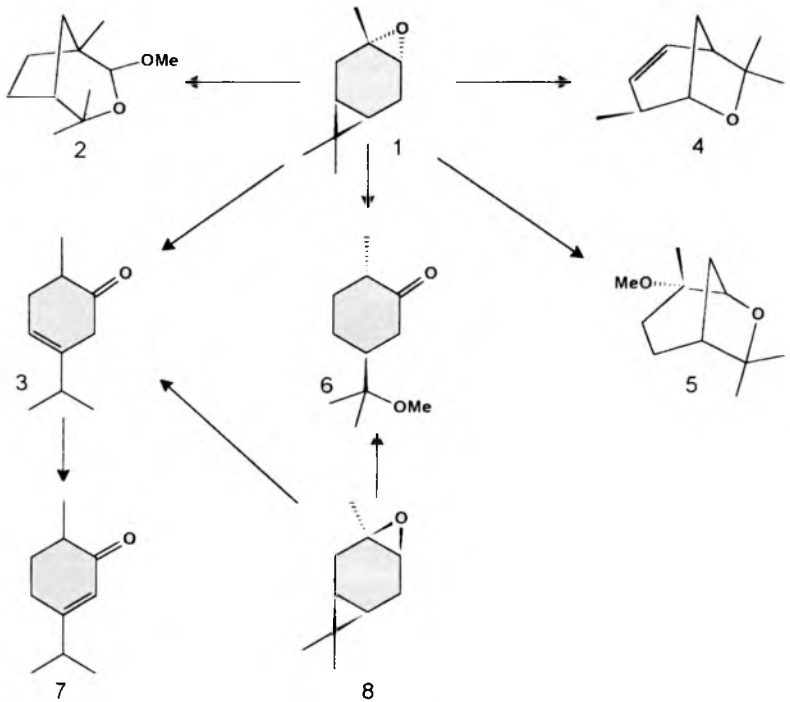
It has been shown that the rearrangements of isomeric 2,3-epoxy caranes in superacid give a different set of products. As can be concluded from Scheme, the configuration of the epoxy cycle affects on the choice of main reaction paths.



For instance, dissolution of isomer 1 in HSO_3F-SO_2FCl system at -110° C followed by "quenching" with a CH_3OH-$(C_2H_5)_2O$ mixture led to a reaction mixture containing predominantly the 2 and 3, respectively 45 and 30% (GLC). Cis-isomer 8 rearranges under the same conditions to produce ketone 3 with ~60% yield (GLC). If reaction mixture in both cases was chromatographed using Al_2O_3 column instead of SiO_2 the carvenone 7 is obtained instead of ketone 3. The structures of 2-6 were established using ^1H and ^{13}C NMR data. These compounds were not obtained earlier by transformations of epoxydes 1 and 8 in weak acids.

REARRANGEMENTS OF AROMADENDRENE AND LEDENE IN ACID-CATALYZED CONDITIONS.

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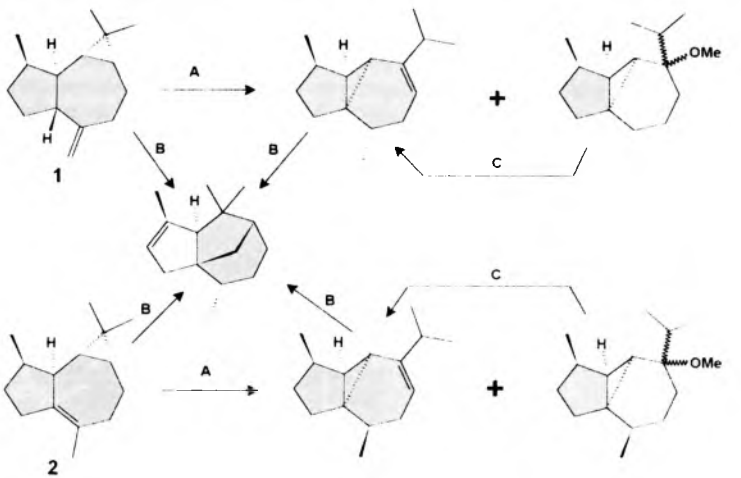
Novosibirsk Institute of Organic Chemistry, Acad. Lavrentjev ave. 9, Novosibirsk, 630090, Russia

For the first time aromadendrene 1 and ledene 2 were transformed in acid-catalyzed reactions to different tricyclic compounds with natural types of skeleton. Their structures were confirmed by ^{1}H and ^{13}C NMR data and the 2D-spectrum of ^{13}C-^{13}C correlation with biquantum coherency (2D-INADEQUATE).



A – HSO_3F-SO_2FCl ;
B – HCO_2H ;
C – SiO_2

The ICAR computer program was used to derive reasonable mechanisms for the acid catalyzed transformations and probability of the mechanisms was estimated using the molecular mechanics method

SYNTHESIS OF CHIRAL PYRAZOLINOLS FROM (+)-3-CARENE.

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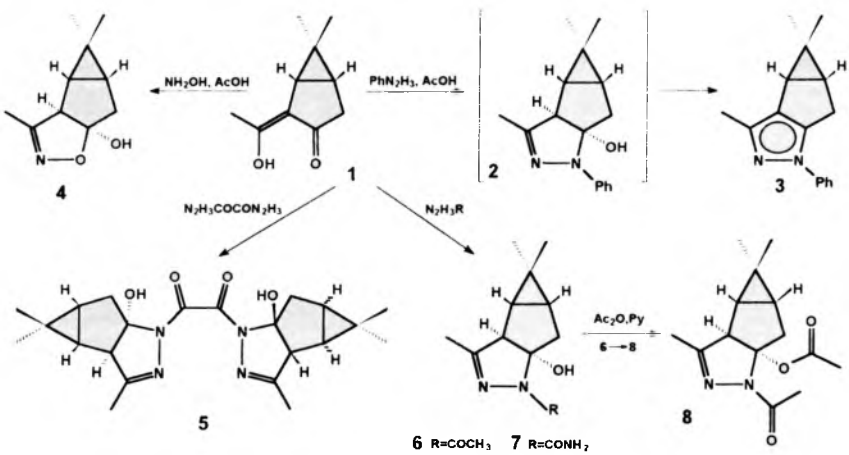
Novosibirsk, 630090, Russia

Reaction of β-diketones with acyl hydrazides is common synthetic route to acyl pyrazoles [1]. We have found that treatment of diketone **1**, derived from monoterpene hydrocarbon (+)-3-carene, with certain acyl hydrazides in boiling methanol in the presence of acetic acid results in formation of stable acyl-2-pyrazolin-5-ols **5-7** rather than corresponding N-acyl pyrazoles. Compounds **5-7** are structural analogs of 2-isoxazolin-5-ol **4**, whose stereochemistry was determined by X-ray crystallography [2]. Our attempts of dehydration of acyl pyrazolinols **5-7** failed. Thus treatment of **6** with acetic anhydride in pyridine affords O-acyl derivative **8**. Tolerance of the acyl pyrazolinols to dehydration results from unfavorable relative position of hydroxyl group and adjacent C-H bonds. At the same time, reaction of **1** with phenyl hydrazine gives no stable intermediates like **2**, only phenyl pyrazole **3** being detected.



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MEVALONATE KINASE ACTIVITY IN SUSPENSION CULTURED CELLS OF *CATHARANTHUS ROSEUS*

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The phosphorylation of mevalonate by mevalonate kinase is an early step in the biosynthetic pathway to terpenoids. As such, this enzyme might well play an important role in the regulation of terpenoid biosynthesis.
Mevalonate kinase activity was studied in a cell culture of *Catharanthus roseus* (L.) G. Don (Apocynaceae). The specific mevalonate kinase activity in *C. roseus* cells cultured on MS medium[1] increased during the exponential phase of the growth cycle, reaching a maximum of 0.5 nkat/mg protein at 4 days after subculturing. After 6 days, which corresponded to the beginning of the stationary phase, the specific activity decreased. Transferring 14-day-old *C. roseus* cells to an induction medium (IM2[2]) resulted in an increase of the specific mevalonate kinase activity to maximally 1.5 nkat/mg protein after 8 days.
Partial purification of mevalonate kinase activity was obtained by acetone precipitation and ion-exchange chromatography on a Q-sepharose column using a linear gradient of KCl; 10% of the activity applied to the column was recovered in two separate peaks. The major peak was applied to a Mono-S column and eluted with a pH gradient; 37% of the activity applied was recovered in three separate peaks. It was found that the enzyme is not stable and that the activity may result from several forms of the enzyme.

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Variations in essential oil yield and composition of *Thymus pulegioides* L.

Felice Senatore

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The biochemical group of plants offering evidence of the largest number of chemical races is that containing volatile oils. Labiatae family comprises about 200 genera and 3300 species; aromatic annual or perennial herbs or undershrubs. Many members of the family are used as culinary or medicinal herbs, as sources of volatile oils many of which are rich in monoterpene alcohols and phenols. Thyme oils rich in phenols have antiseptic and fungicidal activity and are used in the food flavour industry as well as for their medicinal properties. One important factor to be considered in thyme oil production is seasonal variation of oil yield and composition. *Thymus* is noteworthy for the numerous species and varieties of wild growing plants, some of them bearing vernacular names[1-3]. *Thymus pulegioides* L. (*Serpyllum* Section) is a species endemic in the peninsula Sorrentina (Naples, Southern Italy) and could be harvested for commercial production of thyme oil. As a continuation of studies on medicinal plants used in etnomedicine in Southern Italy[4-6] the yield and chemical composition of essential oil obtained by hydrodistillation from leaves of *T. pulegioides* at different harvest time were investigated. Results showed that the highest phenols content coincide with the highest oil yield and that the full flowering is the best time to harvest the plant.

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ALKYLATION OF BENZENE AND ITS MONO- AND DISUBSTITUTED DERIVATIVES WITH CAMPHENE ON WIDE-PORE β-ZEOLITE

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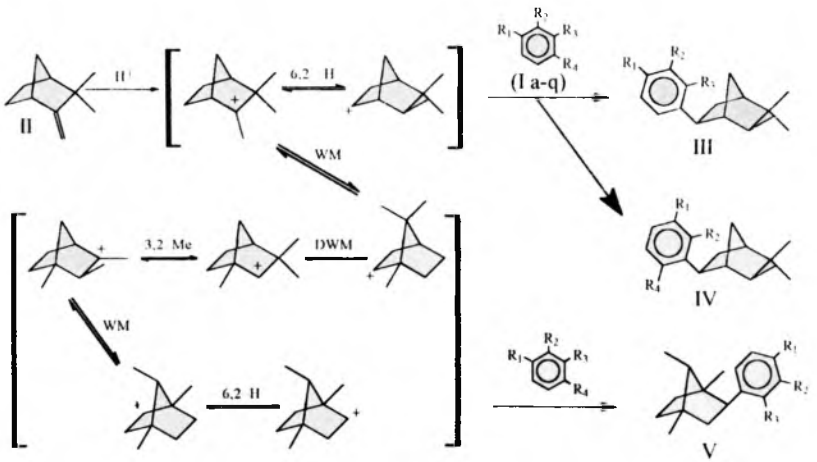
Institute of Organic Chemistry, Siberian Branch RAS, Novosibirsk, Russia

Alkylation of aromatic compounds with olefins or alcohols on zeolite catalysts is widely investigated and usually require high temperature (300-400°C) [1]. We showed that interaction of benzene (Ia) with optical active camphene (II) at room temperature on wide-pore β-zeolite lead practically to the only racemic product (IIIa) with 75% yield.



a: $R_1=R_2=R_3=R_4=H$; b: $R_1=CH_3$, $R_2=R_3=R_4=H$; c: $R_1=C_2H_5$, $R_2=R_3=R_4=H$;
d: $R_1=t\text{-}C_4H_9$, $R_2=R_3=R_4=H$; e: $R_1=R_2=CH_3$, $R_3=R_4=H$; f: $R_1=R_3=CH_3$, $R_2=R_4=H$;
q: $R_1=R_4=CH_3$, $R_2=R_3=H$.

The reactions of toluene (Ib), ethylbenzene (Ic) and t-butylbenzene (Id) with camphene result practically in a set of racemic products (IIIb-Vb), (IIIc-Vc) and (IIId-Vd) correspondingly. The role of zeolite is not only to provide acid catalysis, as olefin does not interact with benzene even on boiling in glacial AcOH in the absence of zeolite.

Attention is engaged to the structure of compounds of type (V), which formation requires deep structural reconstruction of camphene framework.

Introduction of isomeric xylenes (Ie-g) into the reaction is followed by decrease of the reaction rate and camphene conversion into alkylation products, which is probably associated with steric hindrance of alkylation. Alkylation of o-xylene, m-xylene, p-xylene gives compounds (IIIe-Ve), (IIIf-Vf) and (IVq) correspondingly.

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USEFUL ORGANIC MOLECULES *VIA* NITROSOCHLORINATION OF TERPENE HYDROCARBONS

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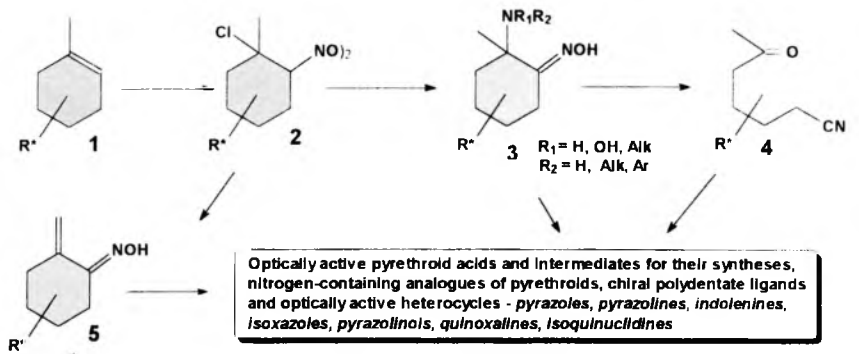
Novosibirsk, 630090, Russia

Among the different types of chiral natural products, terpenes have received the less study as starting materials for the synthesis of optically active heterocyclic compounds. In spite of extremely high enantiomeric purity of some terpenes and their accessibility, the lack of functional groups makes them less attractive as synthetic precursors of heterocycls. We have developed very simple method of functionalization of unsaturated cyclic hydrocarbons **1**, having trisubstituted carbon-carbon double bond, to the corresponding α-amino oximes **3** and *seco*-derivatives: ω-keto nitriles **4**. Nitrosoclorides **2** are the intermediate products in this pathway and can be transformed also to α,β-unsaturated oximes **3**. Nitrosochlorides **2**, derived from terpenic hydrocarbons, are very unstable compounds, so we have designed a simple and handy work-up that makes it possible to operate with these labile compounds without any difficulty. The work-up was found to be easily scalable and was applied at the pilot plant. Using the synthetic pathway developed we have synthesized a number of functionalized derivatives of natural terpenes - 3-carene, α-pinene, limonene, α-muurolene, δ-cadinol, caryopyllene - and used them for the syntheses of optically active heterocyclic compounds having the moiety of pyrazole, pyrazoline, indolenine, isoxazole, pyrazolinol, quinoxaline, isoquinuclidine. The *seco*-derivatives of (+)-3-carene was shown to be useful starting material for the synthesis of a number of optically active pyrethroid precursors - 1*R*- and 1*S*-dihydrochrysanthemolactones, 1*R*-*trans*- and 1*R*-*cis*- chrysanthemic acids, 1*R*-*cis*-nor-chlorochrysanthemic acid, 1*R*-*cis*-permethric and deltamethric acids, 1*R*-cis-chrysanthemylamine and its N-monoalkyl and N,N-dialkyl derivatives together with the pyerthroid aza-analogs. Syntheses of the chiral heterocycles and optically active pyrethroid components are discussed.



A NEW HEPATOPROTECTIVE PREPARATION SALSOCOLLIN FROM SALSOLA COLLINA PALL

Askar H.Tokpaev, Elmira A.Kulmagambetova, Sergazy M.Adekenov, Kairolla D.Rakhimov, Azat A.Abdrakhmanov

Institute of Organic Synthesis and Coal Chemistry, NAS RK, Karaganda, Kazakhstan Scientific and Productional Introduction Small Enterprise "Tabigat", Karaganda State Scientific and Practical Centre "Medstandart", Almaty, Kazakhstan

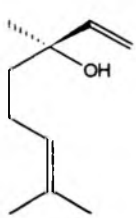
Salsola collina Pall is an annual plant growing in steps, salinas that are spread within CIS, Central and Nothern Kazakhstan. It is used in folk medicine for liver diseases. Hepatoprotective activity of this new natural preparation salsocollin produced on the basis of alcohol extract of *Salsola collina Pall* has been studied under experiment and clinics. Toxicity of preparation in doses of 10000 mg/kg internally and intraperitoneumly (in form of water solution) doesn't cause deathes of mice, rats and rabbits. Preparation doesn't act to general condition, body's weight, hemogram, cardio-vascular system, central nervous system, functional condition of liver and kidneys of rats and dogs. On the ground of these results and data of pathomorphology examination of experimental animals, there have been proved that salsocollin is non-toxic preparation at administration over a long period of time. Through experimental examination there have been proved salsocollin effectively normalizes biochemical indices of liver and considerably inhibits development of pathologic changes of liver, bile and gallbladder. Obtained data acted as a ground for its testing in clinical practice. It seems to be perspective to further investigate the preparation clinically regarding the drug form of its application in clinical practice.

FLORAL FRAGRANCE CHEMISTRY IN THE EARLY FLOWERING SHRUB *DAPHNE MEZEREUM* (THYMELAEACEAE)

Anna-Karin BORG-KARLSON[1], C. Rikard UNELIUS[1], Irena VALTEROVÁ[2] and L. Anders NILSSON[3]

[1]Department of Chemistry, Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden
[2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of The Czech Republic, 166 10 Prague, The Czech Republic
[3]Department of Systematic Botany, Uppsala University, S-752 36 Uppsala, Sweden

The floral fragrance of the early flowering shrub *Daphne mezereum* (Thymelaeaceae) in central Sweden was collected by means of the head-space technique. Gas chromatography-mass spectrometry and a multi-dimensional gas chromatography were used for the analyses and the identifications of the components. (*S*)-(+)-Linalool (I) was the main constituent (95%) of the flower fragrance and its enantiomeric purity exceeded 99% in the samples. The (2*S*,5*S*)- and the (2*R*,5*S*)-furanoid (II) and the (3*R*,6*S*)- and (3*S*,6*S*)-pyranoid (III) linalool oxide isomers constituted 2-5% of the fragrance. The absolute configuration of these four linalool oxides corresponded to that of (S)-(+)-linalool. The elution order of these compounds on a permethylated β-cyclodextrin capillary column is reported. A fragrance sample of *Daphne mezereum* as well as (*S*)-(+)-linalool attracted males of the vernal solitary bee species *Colletes cunicularius* and *Andrena cinerea*. A racemic mixture of the two enantiomeric pairs of known furanoid linalool oxides was only weakly attractive to the bees. The role of the fragrance in the pollination specialization of the plant is discussed.



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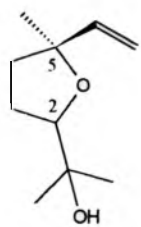

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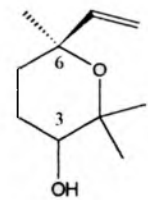


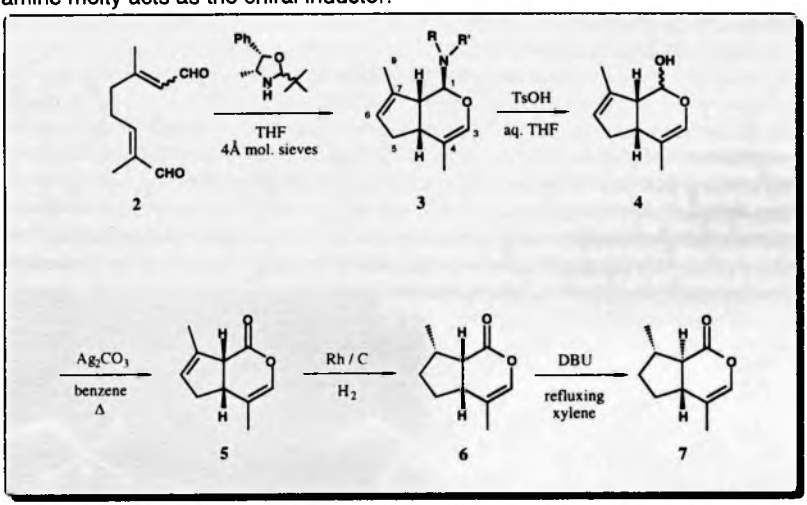
III

A SHORT ASYMMETRIC ROUTE TO IRIDOIDS

Rikard UNELIUS and Lenka CHUMAN

Royal Institute of Technology, Department of Organic Chemistry,
S-100 44 Stockholm, Sweden.

Novel iridoid monoterpenes have been stereoselectively synthesized in 4-5 steps from citral according to the scheme below.[1] The key step is an efficient intramolecular [4+2]-cycloaddition of a enamine derivative of 8-oxocitral, wherein the enamine moity acts as the chiral inductor.[2]



Gastrolactone (**5**) and cis-cis nepetalactone (**II**) are relevant for studies of the chemical communication of chrysomelid beetles[3,4] and aphids[5], respectively. The most important achievement is, however, the synthesis of gastrolactol (**4**), as this compound should be a very useful intermediate in the synthesis of more elaborate iridoids. The double bond in the five-membered ring can be used for further addition reactions or for allylic oxidations or halogenations. A number of iridoids seem to be within reach.

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INFLUENCE OF YEAR SEASON ON FORMATION OF TAXANES IN THE *TAXUS BACCATA* L. BARK.

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Taxol, a highly functionalized diterpene amide, possesses important antitumor and antileukemic activities. Thanks to its unique mechanism of action, it appears to be the prototype of a new class of cancer chemoterapeutic agents (1). The most common source of taxol is the bark of *Taxus brevifolia*, but it is also found in other *Taxus* spp.(2). The objective of this study was screening of taxanes in local species - *Taxus baccata*. In the poster we have described the results of evaluation of the effect of year season on the content of 5 taxanes, previously isolated in our laboratory from *Taxus baccata* bark. Obtained results demonstrated that the season in which samples are collected influences the content of taxanes significantly. The concentration of taxanes was the highest in October and the lowest in January. Notable implication of these results is opportunity for choosing the right season for harvesting of plant material for the purposes of isolation of taxanes.

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STEVIOSIDE PRODUCTION IN THE INTACT AND TUMOUR TRANSFORMED *STEVIA REBAUDIANA* PLANTS.

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Stevia rebaudiana - member of the Asteraceae from Paraguay, is important as a source of the natural swetener, diterpenoide glycoside stevioside (1).
We investigated the presence and amount of stevioside in the leaves of *Stevia* by HPLC. In samples of 37 plants harvested in the autumn 1994 in Czech Republic stevioside is presented between 7.3% and 11.7%. We also discovered content of stevioside in hairy root cultures cultivated in the dark for 4 weeks (2). We used a wild strain of *Agrobacterium rhizogenes* for the infection. The contents of stevioside is lower then 1%. In the future experiments we plan to elucidate, if the amount of stevioside in the hairy root culture is residue from primary explantate or if it is syntetized *de novo*.

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TAXANES IN *TAXUS BACCATA* L. PLANT CELLS

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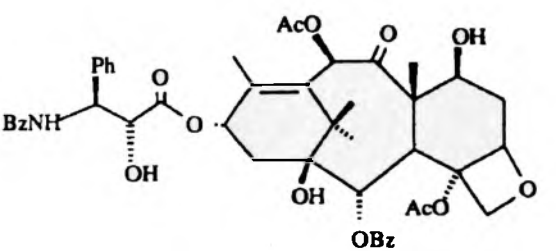
The novel diterpenoid taxol (1) has become one of the most important compounds to emerge from the screening of natural products in recent years. In spite of this promising spectrum of activity, progress on developing taxol as a drug has been slow, largely because of the difficulty of isolating it from the bark of the western yew, *Taxus brevifolia*.



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One possible approach how to solve this problem is utilization of plant tissue cultures, which represent teoretically unlimited source of desired compounds.

In this poster we have described results obtained using callus cultures of *Taxus baccata*. For cultivation of callus cultures we have used the agar media according to Murashige and Skoog supplemented with different combination of growth hormones hormones. According HPLC/DAD, ^{1}H NMR and biological activity tests we were able to identified in various *Taxus baccata* strains (TEB581, TEE6 and TEMS8) 10-Deacetyl-7-xylosyltaxol B, 10-Deacetyl-7-xylosyltaxol C and 10-Deacetyl-taxol. Identification of other taxanes is in progress.

This work was supported by grant AVČR No.555114

NEW ACETYLENIC GERANYL CYCLOHEXENONES FROM *TRICHOLOMA ACERBUM* AND NEW TRICHOAURANTIANE DITERPENOIDS FROM *TRICHOLOMA USTALOIDES*

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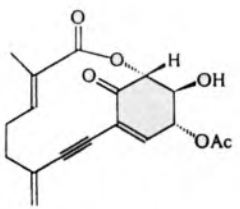
In the course of our studies on *Tricholoma* species (Basidiomycetes),[1] we have examined two EtOAc extracts of *Tricholoma acerbum* (Bull.:Fr.)Quel. and *Tricholoma ustaloides* Romagn. which are considered inedible mushrooms. Five unprecedented acetylenic geranyl cyclohexenone derivatives (e.g. **1-2**) have been isolated from the former mushroom while *T. ustaloides* afforded five new diterpenoid lactones with the recently discovered trichoaurantiane skeleton (e.g. **3-4**).[1]



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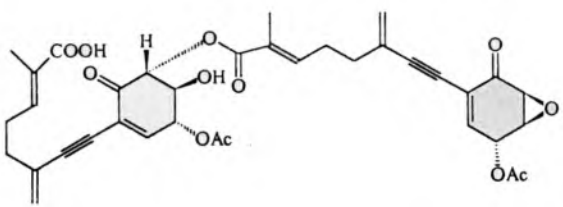

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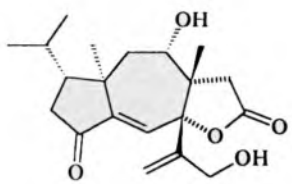

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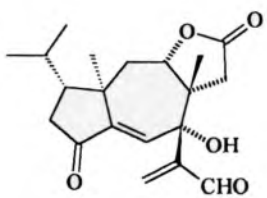

4

The structures were established by 2D-NMR spectroscopy and simple chemical reactions. Compounds of type **1** show a good antibacterial activity against gram-positive bacteria and antimitotic activity towards lymphocyte T cell cultures.

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BIOLOGICALLY ACTIVE SESQUITERPENE LACTONES OF TANACETUM PLANTS

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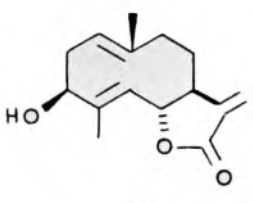
In 13 species of *Tanacetum L.* there have been found presence of 50 sesquiterpene lactones of which 25 were germacranolides, 12-eudesmanolides and 13-guaianolides. Most representatives of this isoprenoids group contain α- methylene γ- lactone function; there are present the ester remains of acetic, angelic acids, epoxide and hydroxyl groups.

We studied 7 species of *Tanacetum L.* from natural flora of Kazakhstan as to the presence of sesquiterpene lactones. Here at, we found out the presence of given compounds in all studied species. There have been isolated and studied the following sesquiterpene lactones: hanphillin (1), tatridin A (2), crispolide (3), tavurin (4) from *Tanacetum vulgare L.*, tamirin (5) from *Tanacetum Karelinii*, tatridin A (2) from *Tanacetum santolina*, tavurin (4), tamirin (5), crispolide (3), hanphillin (1) from *Tanacetum ulutavicum*.

Sesquiterpene lactones have been identified on the base of obtained physico-chemical constants, spectral data (IR, UV, 1 H-NMR, 13 C-NMR) and results of HPLC.

The structures of hanphillin and tamirin have been studied by X-ray method.



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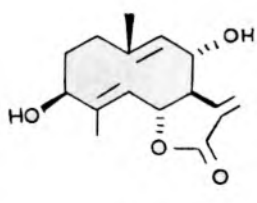

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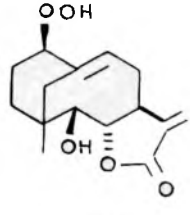

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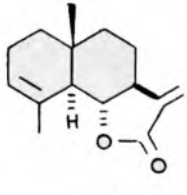

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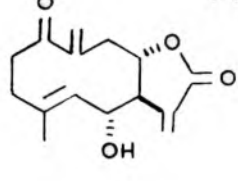

(5)

EMPLOYMENT OF DMSO WITH IODINE FOR OXIDATION OF OLEANOLIC ACID DERIVATIVES

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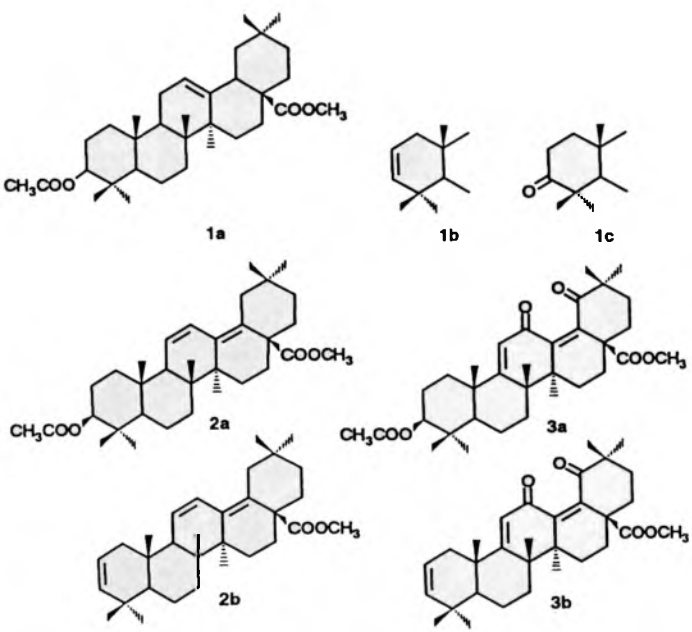
Oxidation of methyl acetyloleanolate **1a** with DMSO in the presence of iodine afforded diene **2a** and conjugated dione **3a** in about 70% overall yield. The corresponding oxidation of methyl olean-2,12-diene-28-oate (**1b**) leads to a mixture of respective compounds **2b** and **3b** in overall yield over 80%. Under these conditions methyl 3-oxoolean-12-ene-28-oate (**1c**) gives a complex mixture of many products.

Compound **3a** was previously obtained by Kon [1] and Ruzicka [2] who used selenium dioxide as an oxidant.

The oxidative properties of DMSO in the presence of iodine have not been reported for triterpenes as yet.

The structures of compounds **2a**, **2b** and **3a**, **3b** were elucidated by spectral methods.



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