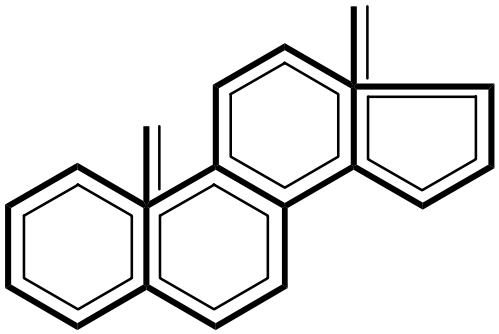
20TH CONFERENCE ON ISOPRENOIDS 2003

LIBEREC, CZECH REPUBLIC

ABSTRACT BOOK



GUEST EDITORS
IRENA VALTEROVÁ, VLADIMÍR POUZAR, PAVEL DRAŠAR

LIBEREC
2003

THE FRANTIŠEK ŠORM LECTURE:

ENANTIOMERISM AND ORGANISMS: WHAT COULD BE CLARIFIED BY SYNTHETIC CHEMISTRY?

KENJI MORI

Emeritus Professor, The University of Tokyo, 1-20-6-1309, Mukogaoka, Bunkyo-ku, Tokyo 113-0023, Japan
E-mail: kjk-mori@arion.ocn.ne.jp

1. The absolute configuration of bioactive natural products can be determined by synthesis and analysis. The stereochemistry of *exo*-brevicomin (the aggregation pheromone of the western pine beetle) was determined in 1974 by its synthesis from (–)-tartaric acid (Fig. 1), and that of plakoside A (an immunosuppressive marine galactosphingolipid) was determined in 2002 by HPLC comparison of its degradation products with synthetic samples (Fig. 2).

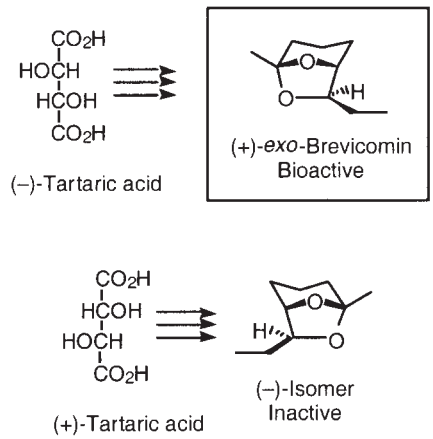


Fig. 1. Synthesis of the enantiomers of *exo*-brevicomin

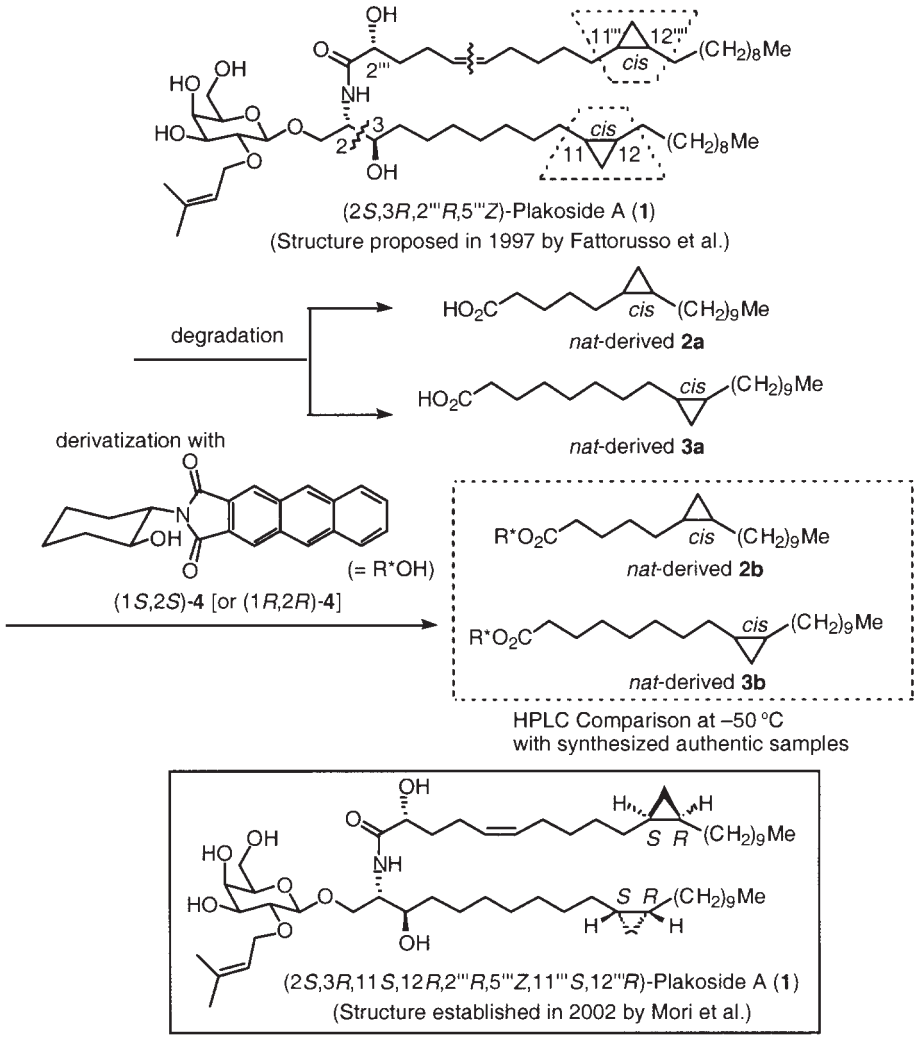


Fig. 2. Determination of the absolute configuration of plakoside A

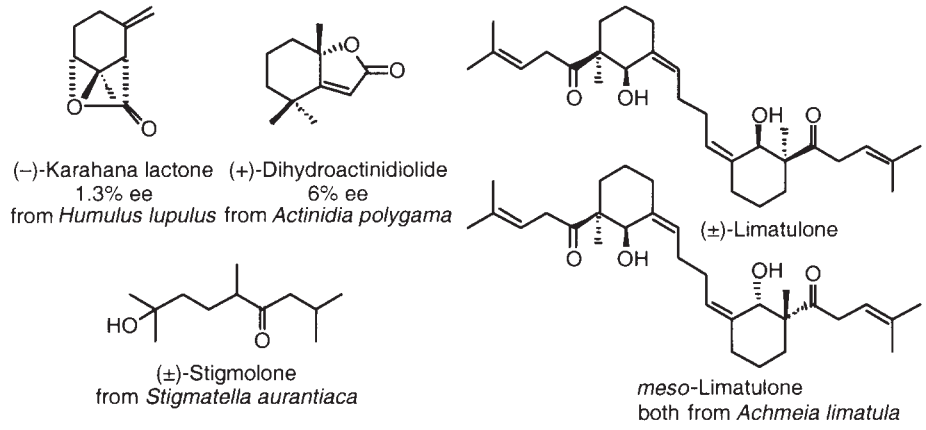


Fig. 3. Examples of natural products which are not enantiomerically pure

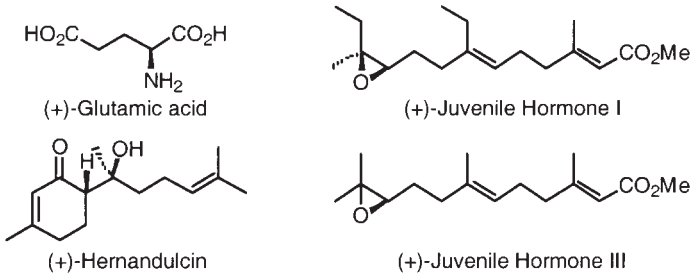


Fig. 4. Only the depicted enantiomers show strong bioactivity

2. A natural product is not always a pure enantiomer. As shown in Fig. 3, natural (–)-karahana lactone and natural (+)-dihydroactinidiolide are almost racemic. The marine defense substance limatulone is biosynthesized by a limpet as a mixture of the racemate and the *meso*-isomer. Stigmolone (the aggregation pheromone of a myxobacterium) is biosynthesized as a racemate.
3. Usually only one of the enantiomers is bioactive (Fig. 4), but sometimes both the enantiomers are active (Fig. 5) as in the cases of nepetalactone (cat attractant) and polygodial (insect antifeedant).
4. Latest enantioselective synthesis of the following new pheromones will be discussed: (4*R*,9*Z*)-9-Octadecen-4-olide (A, the sex pheromone of the currant stem girdler), (*S*)-3,7-dimethyl-2-oxo-6-octene-1,3-diol (B, the aggregation pheromone of the Colorado potato beetle), and (6*S*,19*R*)-6-acetoxy-19--methylnonacosane (C, the sex pheromone of the screwworm fly) (Fig. 6).
5. Stereochemistry-bioactivity relationships among pheromones – Diversity is the keyword of pheromone response. Organisms utilize chirality to enrich and diversify their communication system. The sterochemistry-biochemistry relationships are classified into 10 categories as shown in Fig. 7.
6. Conclusion. Through synthesis we can clarify the diversity in structure-activity relationships, the discovery of which has been the most unexpected and fascinating outcome of our work since I met Prof. F. Šorm in 1970.

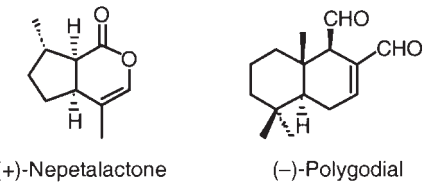


Fig. 5. The depicted enantiomers are natural products, and their opposite enantiomers are also bioactive

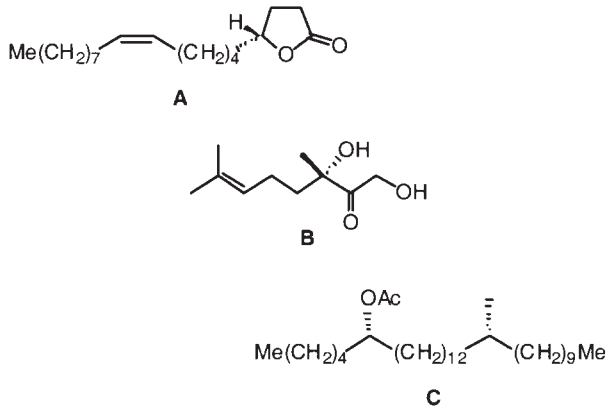


Fig. 6. Structure of new pheromones as synthetic targets

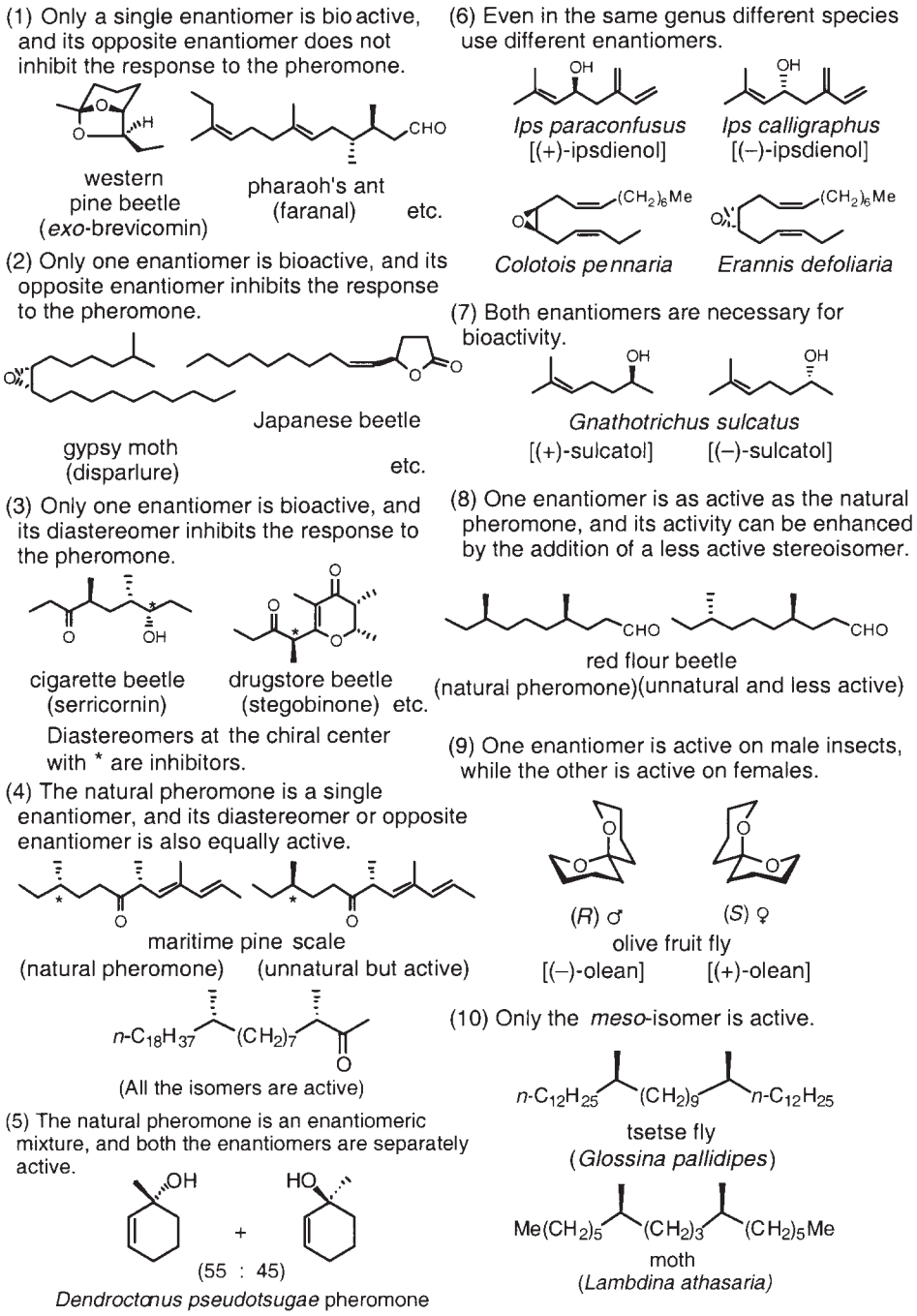


Fig. 7. Stereochemistry and pheromone activity

REFERENCES

1. Mori K.: Acc. Chem. Res. *33*, 102 (2000).
2. Mori K.: Eur. J. Org. Chem. *1998*, 1479.
3. Mori K.: Chirality *10*, 578 (1998).
4. Mori K.: Chem. Commun. *1997*, 1153.
5. Mori K.: Biosci. Biotechnol. Biochem. *60*, 1925 (1996).

SOME FLUORINATED BRASSINOSTEROID DERIVATIVES AT THE A RING. APPROACH TO THE TYPE OF BRASSINOSTEROID--RECEPTOR INTERACTION

MARC AMORÓS, SÒNIA ESPELTA, CARME BROSA*

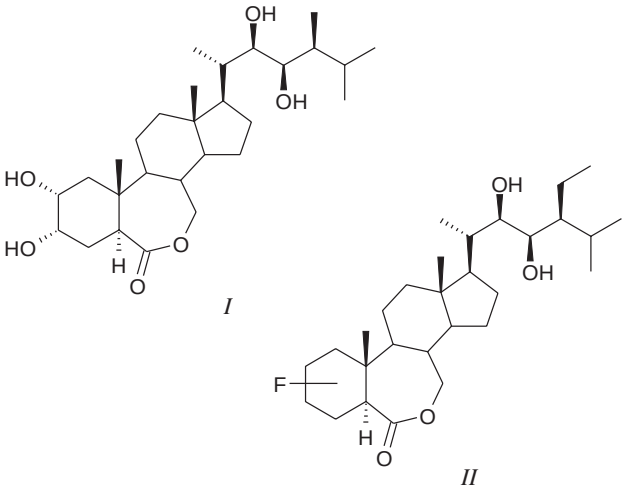
Institut Químic de Sarrià, C.E.T.S., Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain
E-mail: brosa@iqs.url.es

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

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

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

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



I *II*

In this communication, the substitution of some hydroxyl groups at the A-ring by fluor giving brassinosteroid analogs (*II*) will be specifically analyzed from different points of view: molecular modeling, synthesis and bioactivity.

REFERENCES

1. Khripach V. A., Zhabinskii V. N., de Groot A. E.: *Brassinosteroid: A New Class of Plant Hormones*. Academic Press, California 1999.
2. Brosa C.: *Structure-activity Relationship in Brassinosteroids: Steroidal Plant Hormones* (Sakurai A., Yokota T., Clouse S., ed.), Chap. 9. Springer-Verlag, Tokyo 1999.
3. Brosa C., Amorós M., Vàzquez E., Pique M.: Collect. Czech. Chem. Commun. *67*, 19 (2002).

THE CHEMISTRY AND BIOACTIVITY OF BRASSINOSTEROIDS AND THE SEARCH FOR NONSTEROIDAL MIMETICS

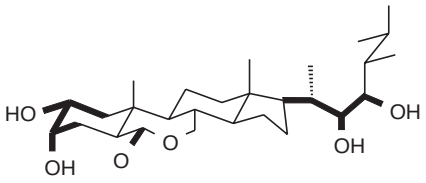
THOMAS G. BACK

Department of Chemistry, University of Calgary, Calgary, Alberta, Canada, T2N 1N4
E-mail: tgback@ucalgary.ca

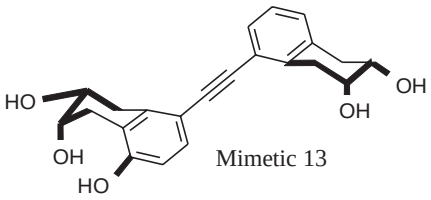
Brassinolide (1) is a remarkably potent phytohormone that has attracted considerable attention since its discovery in 1979 by Grove et al.[1] Although it offers exceptional promise for enhancing the yields of many commercially important crops, its high cost remains a formidable barrier to large-scale applications. Our work on brassinosteroids is directed toward developing more efficient syntheses of brassinosteroids, toward improving our understanding of their structure-activity relationships[2] (SAR) and toward applying this information to the design of cheaper and more efficacious analogues.

The synthesis and bioassay of a series of side-chain and B-ring analogues of 1 have provided us with some fresh insight into their SAR. During this work, two novel compounds 2 and 3 were discovered that have bioactivity exceeding that of 1 by ca. 5–7-fold in the rice leaf lamina inclination bioassay. These products appear to be the most strongly active brassinsoteroids reported to date[3].

A major impediment to commercial exploitation of brassinosteroids stems from their rapid conversion by plants to less active metabolites. Studies elsewhere on the metabolism of brassinosteroids in various species of plants have revealed that



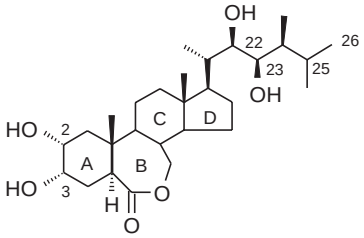
Brassinolide (1)



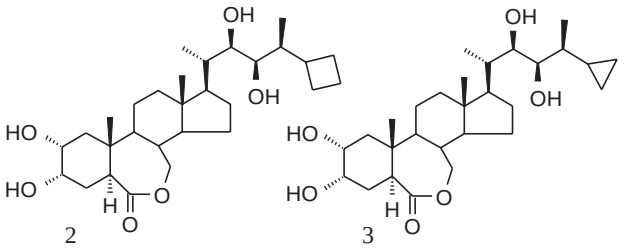
Mimetic 13

Mimetic 14

Fig. 1.

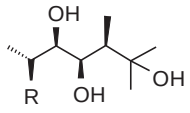


Brassinolide (1)

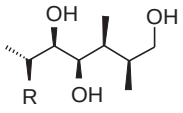


2

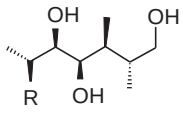
3



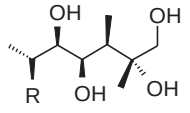
4



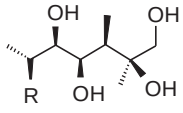
5



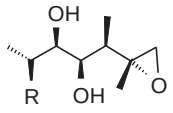
6



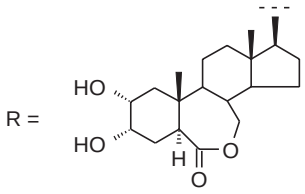
7

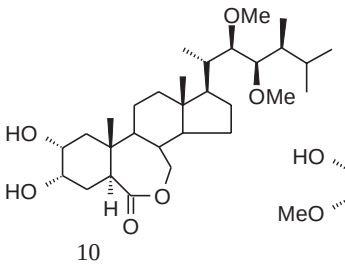


8

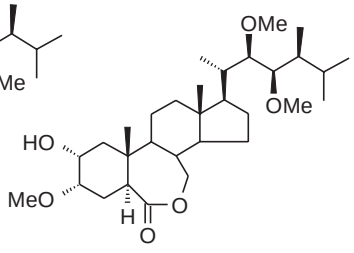


9

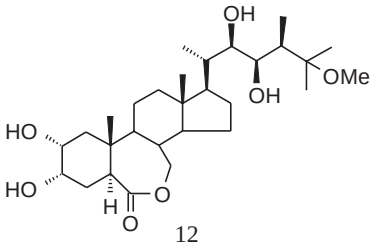




10



11



12

they undergo (inter alia) glucosylation of the hydroxyl groups at C-2, C-3 and C-23, as well as enzymatic hydroxylation, followed by glucosylation at C-25 and C-26 (ref.[4]). While the glucose conjugates appear to be metabolic deactivation products, there has been some controversy regarding the bioactivity of the 25- and 26-hydroxylated aglycones[5]. This has raised the question of whether the formation of these compounds represents an activation or deactivation step. We therefore synthesized all of the stereoisomers of the 25- and 26-hydroxylated brassinolide derivatives (4–8), as well as the epoxide 9. All had very low bioactivity, thereby confirming that the naturally-occurring compounds in this series are deactivation products[6]. We then synthesized a series of *O*-methylated derivatives of brassinosteroids, in the hope of discovering compounds that retain the activity of 1, but where the glucosylation of key hydroxyl groups would be blocked. The methyl ethers 10–12 all displayed relatively strong bioactivity, while other derivatives (e.g. methyl ethers at C-2) were essentially devoid of activity[7]. Methyl ether formation could, in theory, allow brassinolide or its congeners to resist deactivation through glycosylation and thus offer greater persistence in field applications. It also permits us to conclude that free hydroxyl groups are not required at all positions, while at others (e.g. C-2), they are crucial for bioactivity.

Finally, we attempted to design nonsteroidal mimetics of brassinolide that would have simpler structures, thus making them easier and cheaper to synthesize, but that would retain the bioactivity of natural brassinosteroids. Our approach involved the use of subunits containing the essential structural features of brassinosteroids (namely the two vicinal diol units and a polar functional group on the B-ring) held in the correct spatial orientation. The subunits were then joined by rigid linkers designed to hold the subunits the required distance apart. A series of mimetics that had structures capable of superimposing closely with that of brassinolide (1) were identified and synthesized[8]. Two of the mimetics (13 and 14; see Fig. 1; essential structural features are shown in bold) showed significant bioactivity when synergized by the auxin IAA. When applied under optimum conditions, 14 proved comparable to 1 in the rice leaf lamina inclination bioassay. The relatively simple and symmetrical structure of such mimetics may prove advantageous with respect to the cost of their production.

REFERENCES

1. Grove M. D., Spencer G. F., Rohwedder W. K., Mandava N., Worley J. F., Warthen Jr. J. D., Steffens G. L., Flippen-Anderson J. L., Cook Jr. J. C.: Nature *281*, 216 (1979).
2. For recent reviews, see: (a) Brosa C., in: *Brassinosteroids: Steroidal Plant Hormones* (Sakurai A., Yokota T., Clouse S. D., ed.), pp. 191–222. Springer-Verlag, Tokyo 1999; (b) Khripach V. A., Zhabinskii V. N., de Groot A. E., in: *Brassinosteroids: A New Class of Plant Hormones*, pp. 301–324. Academic Press, San Diego 1999; (c) Yokota T., Mori K., in: *Molecular Structure and Biological Activity of Steroids* (Bohl M, Duax W. L., ed.), pp. 317–340. CRC Press, Boca Raton 1992.
3. Back T. G., Janzen L., Nakajima S. K., Pharis R. P.: J. Org. Chem. *65*, 3047 (2000).
4. For recent reviews, see: (a) Adam G., Schneider B., in:

Brassinosteroids: Steroidal Plant Hormones (Sakurai A., Yokota T., Clouse S. D., ed.), pp. 113–136. Springer-Verlag, Tokyo 1999; (b) ref. 2b, pp. 55–72.
5. (a) Adam G., Porzel A., Schmidt J., Schneider B., Voigt B., in: Studies in Natural Products Chemistry (Atta-ur--Rahman, ed.), Vol. 18, pp. 495–549. Elsevier, Amsterdam 1996; (b) Hai T., Schneider B., Adam G.: Phytochemistry *40*, 443 (1995); (c) Voigt B., Porzel A., Golsch D., Adam W., Adam G.: Tetrahedron *52*, 10653 (1996); (d) Seto H., Fujioka S., Koshino H., Yoshida S., Tsubuki M., Honda T.: Tetrahedron *55*, 8341 (1999).
6. Pharis R. P., Janzen L., Nakajima S. K., Zhu J., Back T. G.: Phytochemistry *58*, 1043 (2001).
7. Back T. G., Janzen L., Pharis R. P., Yan Z.: Phytochemistry *59*, 627 (2002).
8. Andersen D. L., Back T. G., Janzen L., Michalak K., Pharis R. P., Sung G. C. Y.: J. Org. Chem. *66*, 7129 (2001).

INFLUENCE OF BRASSINOSTEROID BIOSYNTHESIS INHIBITOR, Brz2001, ON *Chlorella vulgaris*

ANDRZEJ BAJGUZ[a*], TADAO ASAMI[b]

[a]*University of Bialystok, Institute of Biology, Swierkowa 20 B, 15-950 Bialystok, Poland,* [b]*RIKEN, 2-1 Hirosawa, Wako, Saitama, 351-0198 Japan*
E-mail: abajguz@uwb.edu.pl

Brassinosteroids (BR) are natural plant steroidal compounds that promote growth and affect a broad spectrum of physiological responses at nanomolar to micromolar concentrations. They have an essential role in plant development, which have given these compounds the status of plant hormones. Subsequently about 60 related compounds from plants were shown to have BR activity[1–3]. Inhibitors of the biosynthesis and metabolism of BR have crucial roles in analysing the functions of BR in plants. The first specific BR biosynthesis inhibitor, brassinazole (Brz) was discovered. Brz, a triazole derivative, inhibits the plant growth, which is recovered by application of brassinolide (BL). Brz blocks the conversion of 6-oxocampestanol to cathasterone (CT), CT to teasterone (TE), campestanol to 6-deoxoCT, 6-deoxoCT to 6-deoxoTE in BR biosynthetic pathways[4,5].

In this study, Brz applied as a diastereomeric mixture of Brz2001, acts as a growth inhibitor of light-grown *Chlorella vulgaris* cells. The effects of Brz2001 on the metabolism of light-grown algae are also reported. Brz2001 exhibited a stimulative effect on the growth of cells in darkness. Co-application of BL with Brz2001 results in normal growth in both light- and dark-grown cells. However, treatment with BL and Brz2001 stimulates growth more strongly in light-grown cells than in dark-grown cells. BL was used at the concentration of 10 nM, which has the greatest effect on the growth and metabolism in light-grown cells.

Addition of 0.1–10 μM Brz2001 to light-grown *C. vulgaris* cells inhibits their growth during the first 48 h of cultivation. However, treatment with Brz2001 at concentrations lower than 0.1 μM results in growth levels very similar to that of control cell. Brz2001 most inhibited *C. vulgaris* cell growth at 0.5–10 μM, during the first 36 h of cultivation in the light, with a concentration of 10 μM Brz2001 showing the greatest growth-inhibitory effect. During the next 12 h, Brz2001 had no effect on growth. During the first 36 h, a treatment of cultured cells with both 0.1–1 μM Brz2001 and BL showed a weaker stimulative effect than did a treatment with Brz2001 alone. Treatment with a higher concentration of Brz2001 (5 or 10 μM) plus BL resulted in weaker suppression of growth. However, the combined treatment with Brz2001 and BL appeared to have a stimulative effect on the number of cells at 48 h, as compared to the control. We postulate that the growth retardation of Brz2001-treated cells is caused by the BR deficiency of the cells, and therefore this retardation can be reversed by the addition of BL to light-grown cells.

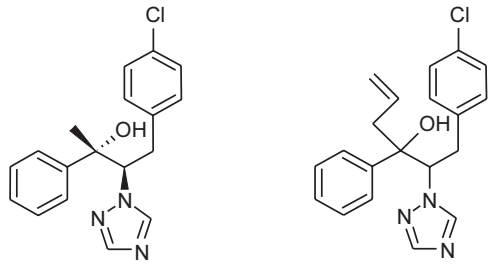
Brz2001 has a dual influence on the metabolism of *C. vulgaris* in the light, which varies with its concentration and the length of cultivation. First, it has an early inhibitory effect on the contents of RNA (12–24 h: 0.1–10 μM; 36 h: 5–10 μM), protein (12–24 h: 0.1–10 μM), chlorophylls and carotenoids (12–36 h: 0.1–10 μM; 48 h: 5–10 μM) and sugar (12–36 h: 0.1–10 μM). Later, it has a stimulatory effect on the contents of RNA (36 h: 0.1–1 μM; 48 h: 0.1–10 μM), protein (36–48 h: 0.1–10 μM), chlorophylls and carotenoids (48 h: 0.1–1 μM) and sugar (48 h: 0.1–10 μM). The interaction of Brz2001 and BL showed only a stimulatory effect on the metabolism of *C. vulgaris* during a period of up to 48 h of cultivation in the light.

In contrast to inhibiting the growth of light-grown cells, Brz2001 promoted the growth of dark-grown cells, especially at 10 μM, with maximal stimulation between 12 and 24 h. Application of both Brz2001 and BL to dark-grown cells resulted in stimulation of growth as compared to non-BL-treated cells, but in less stimulation of growth than in cells treated with Brz2001 alone. Dark-grown cells treated with BL showed a slight growth stimulation at 24 h. During the next consecutive 24 h of cultivation, the BL-treated cells underwent complete stagnation. This is the first evidence that the combination of BR and light has an essential role in the normal growth of *C. vulgaris*. It was also found that dark-grown cells treated with 10 μM Brz2001 are more active biologically than cells treated with BL and 0.1 μM Brz2001.

Finally, Brz2001 blocked algal growth and metabolism, especially during the first 24 h of cultivation, and this blockage was reversed by the co-application of BL with Brz2001. The blockage of BR biosynthesis[4,5] and the suppression of growth and metabolism of this alga by Brz indicate the essential role of BR in algal development in the light. It is probable that the presence of endogenous BR during the initial steps of the *C. vulgaris* cell cycle is indispensable to their normal growth and development in the light.

The precursor of BR is campesterol, one of the major plant sterols, which is primarily derived from isopentenyl diphosphate (IPP). IPP is synthesized from acetyl-CoA *via* mevalonic acid (mevalonate pathway) or by pyruvate and glyceraldehyde 3-phosphate (non-mevalonate pathway). Many vascular plants use both of these pathways to synthesize sterols[6,7]. Various BR biosynthetic intermediates have been identified in *Arabidopsis thaliana* and *Catharanthus roseus*. These results are helping to elucidate the boundary between sterols and BRs, beginning with the mevalonate pathway[2,8,9].

We have investigated the origin of BR in *C. vulgaris* by testing the effects of a mevalonate pathway inhibitor (mevi-



brassinazole (Brz) Brz2001

Fig. 1. Structures of brassinazole derivatives

nolin, Mev) and a non-mevalonate pathway inhibitor (clomazone, Clo) on its growth. Mev had no effect, but Clo caused growth inhibition that could be rescued by BL. This result suggests that in algae, only the non-mevalonate pathway may exist, and that the BR in algae must be synthesized through the non-mevalonate pathway.

REFERENCES

1. Bajguz A., Tretyn A.: Phytochemistry *62*, 1027 (2003).
2. Bishop G. J., Yokota T.: Plant Cell Physiol. *42*, 114 (2001).
3. Zullo M. A. T., Kohout L., de Azevedo M. B. M.: Plant Growth Regul. *39*, 1 (2003).
4. Asami T., Min Y. K., Nagata N., Yamagishi K., Takatsuto S., Fujioka S., Murofushi N., Yamaguchi I., Yoshida S.: Plant Physiol. *123*, 93 (2000).
5. Asami T., Yoshida S.: Trends Plant Sci. *4*, 348 (1999).
6. Lichtenthaler H. K.: Annu. Rev. Plant Physiol. Plant Mol. Biol. *50*, 47 (1999).
7. Lichtenthaler H. K.: Biochem. Soc. Trans. *28*, 785 (2000).
8. Fujioka S., Noguchi T., Watanabe T., Takatsuto S., Yoshida S.: Phytochemistry *53*, 549 (2000).
9. Fujioka S., Takatsuto S., Yoshida S.: Plant Physiol. 130, 930 (2002).

BIOCHEMICAL ACTIVITY OF BIOCHANIN A IN *Wolffia arrhiza* (LEMNACEAE)

ROMUALD CZERPAK, ALICJA PIOTROWSKA, ANDRZEJ BAJGUZ, EDYTA CHILIMONIUK

Institute of Biology, University of Bialystok, Swierkowa 20 B, 15-950 Bialystok, Poland
E-mail: alicjap@uwb.edu.pl

Biochanin A (5,7-dihydroxy-4'-methoxyisoflavone) is an isoflavonoid compound characterised by estrogenic activity in humans and animals. It commonly occurs in many species of vascular plants, mostly belonging to the families Papilionaceae and Graminae. Red clover (*Trifolium pratense*), containing of 0,8 % this compound in dry weight of leaves, seems to be the richest source of biochanin A.

In many plant species isoflavonoids can act as phytoncides or phytoalexins, protecting them from the invasion of viral, bacterial and fungial pathogens. The numerous studies carried on mammals' show that biochanin A as well as other isoflavonoids possesses high estrogenic activity. Estrogenic activity of isoflavonoid depends mainly on the number and localization of hydroxyl and methoxyl groups in aromatic skeleton of isoflavone which bears analogical cyclical structure resemblance to estrone, an endogenous steroidal hormone or to dietylostilbestrol – its chemical synthetic analogue. Therefore, *in vivo* biochanin A can interfere with mechanism controlled by animal steroidal hormone through competition for its receptors. In animals cells isoflavonoid compounds play the significant role in regulation of protein phosphorylation, inducing the activity of protein – tyrosine phosphatase and inhibiting the activity of protein – tyrosine kinase. Isoflavonoids are also observed to induce apoptosis in variety types of cancer cells. In liver microsomes these compounds may stimulate the increase of the content and activity of cytochrome P-450.

There is no empirical data concerning biochemical activity of isoflavonoids in vascular plants in which these compounds are commonly present as natural secondary metabolites. In this view, the influence of biochanin A at the range of concentrations 10^{-8}–10^{-5} M on the growth and metabolism of *Wolffia arrhiza* (Lemnaceae), was investigated.

Wolffia arrhiza is the smallest vascular plant, which – depending on environmental conditions, can be a photoautotroph, mixotroph or absolutely heterotroph. In Polish waters it is becoming more and more popular, especially in small and shallow eutrophic reservoirs, rich in organic substances. Owing to this propriety, *Wolffia arrhiza* is used in biological refinery of sew ages. The culture of *Wolffia arrhiza* was performed for the period of 20 days under controlled conditions at 25±0.5 ˚C with 12 hours' long – lighting of photosynthetic active radiation of 50 $\mu M.m^{-2}.s^{-1}$. Plants were grown in glass crystallisers, including 1l of tap water which is rich in mineral elements and poor in organic substances. During experiment, changes in the content of water–soluble proteins, reducing sugars, nucleic acids, chlorophyll *a* and *b*, phaeophytin *a* and *b*, total carotenoids in biomass of *Wolffia arrhiza* were analysed. Concentration of soluble protein was determined by the method of Lowry et al. (1951). The content of nucleic acids (DNA and RNA) was determined spectrophotometrically, according to the method described by Sambrook et al. (1989). The reducing sugar concentration was determined spectrophotometrically, according the Somogyi method (1954) and photosynthetic pigments content in the biomass of *Wolffia arrhiza* – according to the equations of Wellburn (1994).

Results of conducted studies indicated that biochanin A exhibited weak stimulative effect on the contents of biochemical parameters in *Wolffia arrhiza*. Under the influence of 10^{-6} M biochanin A, the contents of chlorophyll *a* and *b* underwent the strongest stimulation between the 15th and the 20th day of cultivation, reaching the maximal values for chlorophyll *a* – 113.1 % and *b* – 124.1 %, in relation to the control culture, non – biochanin A – treated (100 %). On the other hand, biochanin A at the concentration of 10^{-5} M had the most stimulative effect on the content of phaeophytin *a* and *b* (immediate precursors of chlorophylls). On the 5th day of experiment the content of phaeophytin *a* increased to the level of 125.8 % and phaeophytin *b* to – 114.4 %. From among photosynthetic pigments, the highest accumulation of the total content of carotenoids, chiefly under the influence of 10^{-8} M biochanin A, was observed evenly during the whole period of *Wolffia arrhiza* cultivation, reaching the values range

of 118.9–130.2 %, in comparison with the control culture (100 %). The greatest stimulation of nucleic acids contents (DNA and RNA) was observed on the 10th and on the 20th day of cultivation, considerably weaker on the 5th day and the smallest – on the 15th day. The maximum increase in the nucleic acids accumulation in the biomass of *Wolffia arrhiza* was found on the level of 109.9–126.1 % under influence of 10^{-7} M biochanin A. At the concentration of 10^{-6} M biochanin A was found to have the greatest stimulative effect on the content of water–soluble proteins fraction. Protein concentration in the biomass of *Wolffia arrhiza* increased in the range of 107.0–119.9 % between the 5th and the 15th day of culture and to 119.7 % on the 20th day of experiment, in relation to the control culture (100 %). Among all analysed biochemical parameters, the content of reducing sugars was characterised by the highest increase under the influence of 10^{-8} M biochanin A, mainly between the 5th and the 10th day of culture, reaching maximum values: 136.5 % on the 5th day and 156.0 % on the 10th day of cultivation when compared with the control (100 %). The effectiveness of the action of biochanin A on the analysed biochemical parameters depends on the value of concentration of growth factor, the period of its activity and development phase of the whole plant. Results of presented study indicated that stimulative effect of biochanin A on the growth and physiological-metabolic processes of *Wolffia arrhiza* – untipical, photosynthetic vascular plant – wasn't significant. It should be expected that other isoflavonoids like daidzein and genistein, which don't possess methoxyl group (-OCH_3) in the B ring of the isoflavonoid structure, could be more active in the vascular plants in comparison with biochanin A.

REFERENCES

1. Alhasan S. A., Ensley J. F., Sarkar F. H.: Internat. J. Oncol. *16*, 333 (2000).
2. Czerpak R., Piotrowska A., Wierzbowska M.: Pol. J. Environ. Stud. *12*, 163 (2003).
3. Liggins J., Bluck L. J. C., Runswick S., Atkinson C., Coward W. A., Bingham S. A.: J. Nutr. Biochem. *11*, 326 (2000).
4. Lowry P., Rosebrough N. J., Furr A. L., Randall P. J.: J. Biol. Chem. *193*, 265 (1951).
5. Sambrook J., Fritsch E. F., Maniatis T.: CSH Lab. Press (1989).
6. Somogyi M.: J. Biol. Chem. *195*, 19 (1954).
7. Tolleson W. H., Doerge D. R., Churchwell M. J., Marques M. M., Roberts D. W.: J. Agric. Food Chem. *50*, 4783 (2002).
8. Wellburn A. R.: J. Plant Physiol. *144*, 307 (1994).

BRASSINOSTEROIDS: DEEPING MORE ON STRUCTURE-ACTIVITY RELATIONSHIP

CARME BROSA

Institut Químic de Sarrià, C.E.T.S., Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain
E-mail: brosa@iqs.url.es

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

The results reported by applying brassinosteroids over several plants have successfully demonstrated the suitability of such compounds for the enhanced production of field crops and vegetables. Although these findings are encouraging, further detailed studies are required before the full potential use of brassinosteroids can be realized.

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

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

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

Based on molecular modeling techniques and GRID methodology over the set of brassinosteroids studied the good correlation observed between the feasibility of H-bonding and the activity suggests that this type of interaction may take place in the brassinosteroid-receptor complex and a very good QSAR model is obtained. Moreover, considering our finding when the KM-01, the first brassinolide-inhibitor known at the moment, is involved in the study, the results obtained at present have allowed us to provide information about the areas of the molecule responsible for eliciting activity and those only responsible for binding with the receptor.

The assessment of this information and the clarification of how the functionalities present in a brassinosteroid could work on binding through hydrogen bonding with the receptor has become a priority aim in our group. Thus, the interesting points to be determined at present are: (*1*) whether the OH groups

present in an active brassinosteroid act as acceptor or as donor in such hydrogen bonding, (*2*) the contribution of each OH group presents in the brassinosteroid to develop biological activity. Thus, the substitution of the hydroxyl group present in a defined position of an active brassinosteroid by other functionalities acting as acceptor or as donor of H-bonding and the activity evaluation of such compounds should give us more information about the type of interaction that could take place on binding in each of the positions studies.

In this lecture, the results obtained by our group after analyzing several new analogs having other functionalities than OH at different parts of the molecule will be specifically analyzed from different point of view: molecular modeling, synthesis and bioactivity.

REFERENCES

1. Brosa C.: *Biological Effects of Brassinosteroids*, in: *Biochemistry and Function of Sterols* (Parish E. J., Nes D., ed.), Chap. 15, pp. 201–220. CRC Press, Boca Raton 1997.
2. Brosa C.: *Structure-activity Relationship*, in *Brassinosteroids: Steroidal Plant Hormones* (Sakurai A., Yokota T., Clouse S. D., ed.), Chap. 9, pp. 191–222. Springer-Verlag, Tokyo 1999.
3. Brosa C., Nusimovich S., Peracaula R.: Steroids *59*, 463 (1994).
4. Brosa C., Zamora I., Capdevila J. M.: Tetrahedron *52*, 2435 (1996).
5. Brosa C., Puig R., Comas X.: Steroids *61*, 540 (1996).
6. Brosa C., Soca L., Terricabras E., Ferrer J. C.: Tetrahedron *54*, 12337 (1998).
7. Goodford P. J.: J. Med. Chem. *28*, 849 (1985).
8. Wade R. C., Clark K. J., Goodford P. J.: J. Med. Chem. *36*, 140 (1993).

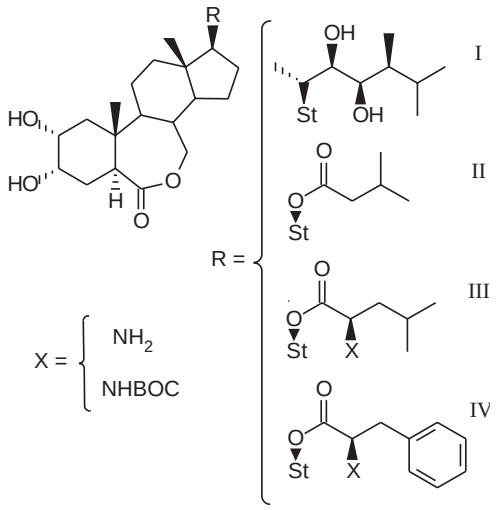
THE EFECT ON ACTIVITY OF A NITROGENATED FUNCTIONALITY IN ANDROSTANE BRASSINOSTEROID ANALOGS

CARME BROSA[a*], LAURA TOBELLA[a], LADISLAV KOHOUT[b], PEP RAYÓ[a], MARC VILAPLANA[a]

[a]Institut Químic de Sarrià, C.E.T.S., Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain, [b]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: kohout@marilyn.uochb.cas.cz, brosa@iqs.url.es

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

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



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

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

New analogs III and IV with an extra NH_2 group protected or not with BOC will be specifically analyzed in this communication. The synthetic strategy developed to obtain them, their feasibility of hydrogen-bonding by means of Grid maps, as well as their activity data will be presented and compared with other analogs.

The work was supported by the grants Nos. 203/01/0083 (GA CR) and Z4 055 905.

REFERENCES

1. Brosa C.: *Structure-activity Relationship*, in: *Brassino-*

steroid: Steroidal Plant Hormones (Sakurai A., Yokota T., Clouse S., ed.), Chap. 9. Springer-Verlag, Tokyo 1999.
2. Goodford P. J.: J. Med. Chem. *28*, 849 (1985).
3. Wade R. C., Clark K. J., Goodford P. J.: J. Med. Chem. *36*, 140 (1993).
4. Brosa C., Zamora I., Terricabras E., Kohout L.: Collect. Czech. Chem. Commun. *63*, 1635 (1998).
5. Kohout L.: Collect. Czech. Chem. Commun. *54*, 3348 (1989).

ADVENTURES IN VITAMIN D CHEMISTRY

MARTIN J. CALVERLEY

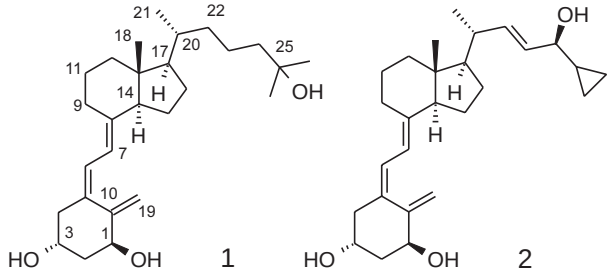
Department of Medicinal Chemistry, LEO Pharma, Industriparken 55, DK-2750 Ballerup, Denmark
E-mail: martin.calverley@leo-pharma.com

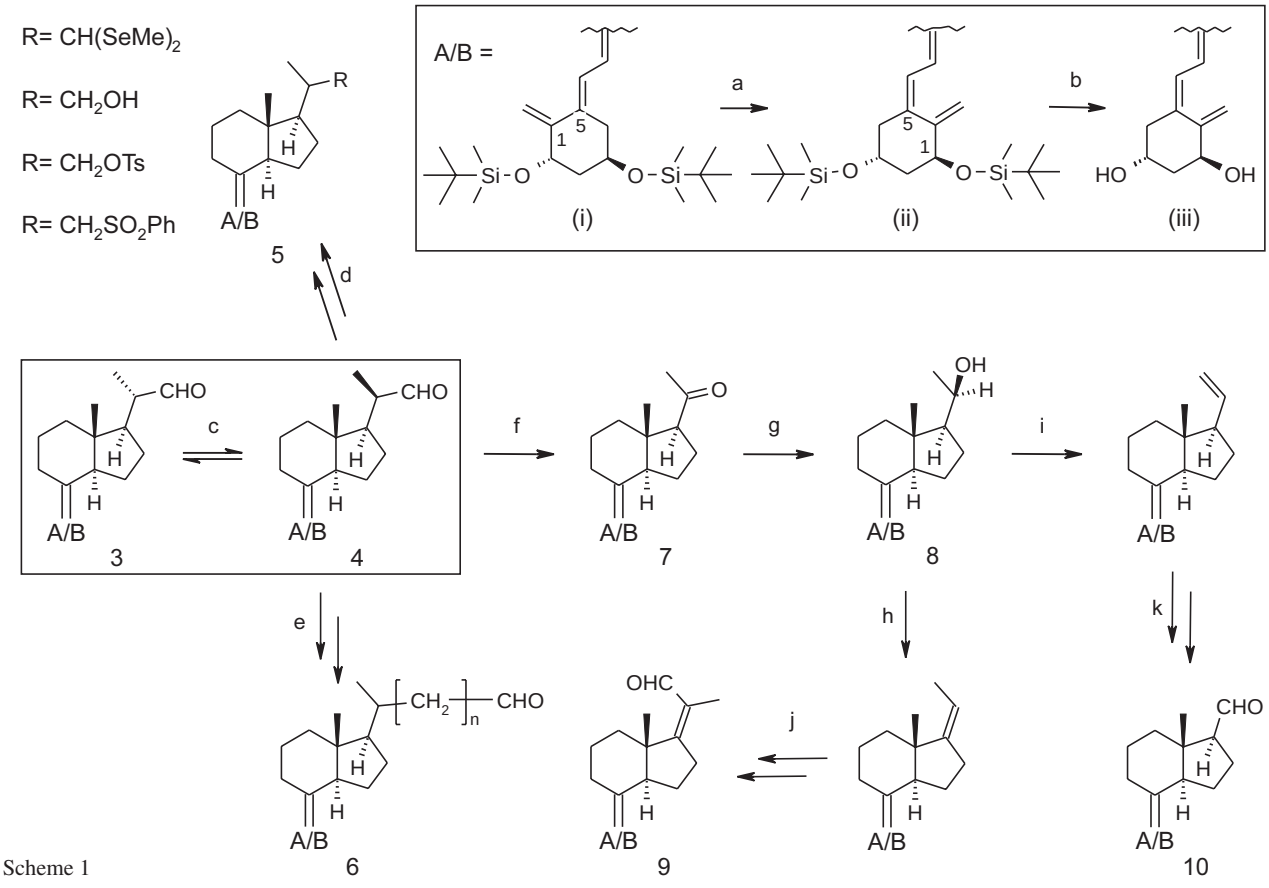
Capitalising on the availability of the C_{22}-skeleton key intermediate (3(i))[1] available in eight steps from vitamin D_2, and an intermediate in the production of the LEO anti-psoriasis drug, calcipotriol (2, an analogue of the natural vitamin D hormone, 1α,25-dihydroxy-vitamin D_3, or calcitriol, 1), we have developed a battery of secondary key intermediates, including the numbered compounds in the silylated series shown in the Scheme 1 (ref[2]).

Although standard reactions were generally employed, the preparation and/or use of some these intermediates have frequently led to unexpected adventures in vitamin D chemistry. Here are a few examples.

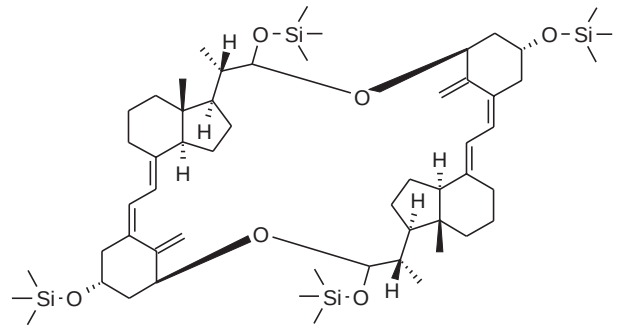
The propensity of the aldehyde 3(i) (or its 5*Z* isomer, 3(ii)) to epimerise at C-20 under basic or acidic conditions has had interesting consequences. The product of desilylation of 3(ii) with a HF mixture, a process that gives rise to the desired 3(iii) plus some epimer 4(iii), was reprotected for purification as its more easily hydrolysable bis-TMS ether: this went according to plan, but was accompanied by the formation of a symmetrical head-to-tail acetal dimer 11. This compound is particularly interesting NMR-spectroscopically, e.g. it presents locked ring-A conformations, with axial 1α and equatorial 3β substituents.

An early investigation of by-products from the lithio-deselenation and subsequent alkylation of the seleno-acetal 20*S*-5(i) (R = $CH(SeMe)_2$) showed that the 1α-silyloxy group under-

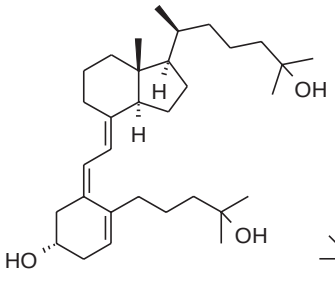




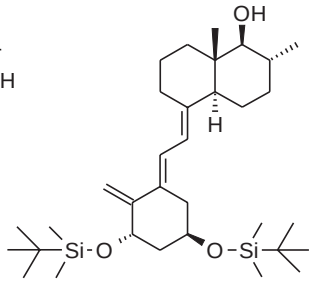
Scheme 1



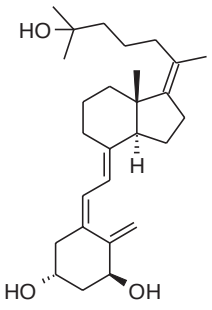
11



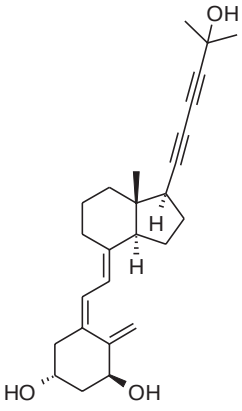
12



13



14



15

went a facile allylic substitution by an excess of *n*-butyl-lithium on warming to room temperature; the result was an "iso--vitamin D" derivative. Furthermore, in model compounds (having just the D_2 or D_3 side chain), "hydride" from LAH or Redal could also be employed as the nucleophile in the S_N2' reaction under suitable conditions. It was even found that the allylic substitution was a significant side reaction in the Li_2CuCl_4-mediated Grignard couplings with for example the tosylate 20*R*-5(i) (R = CH_2OTs). The 5*E* compounds were transformed into compounds of the hitherto undescribed 5*Z*--iso-vitamin D series by triplet-sensitised (fluorenone) photoisomerisation, and finally some of these were deprotected: see for example compound 12.

The reduction products of the 20-ketone 7(i), in addition to being key intermediates in their own right ($NaBH_4$ reduction gives mainly the 20*R* alcohol 8(i), *O*-alkylation of which provides the side chain for the extremely potent 20-epi-22--oxa-compound, KH1060) (ref.[6]), were used to provide (A) the aldehyde 9(i) for an entry into the 17(20)-ene series, and (B) the C_{20}-skeleton 17-carboxaldehyde 10(i), by taking advantage of the complementary regioselectivities of the $POCl_3$ (this also stereoselective) and Martin's sulphurane mediated dehydration reactions (steps h and i, respectively, on the Scheme 1). While only a trace of the 17(20)-ene was produced in the sulphurane reaction, a new by-product was shown to be the D-homo vitamin D compound 13. (Interestingly, the corresponding D-homo rearrangement was not observed in the dehydration of the 20*S*-isomer (not shown) of alcohol 8(i).) The remaining steps in the synthesis of 10(i) from the terminal olefin were masking of the vitamin D triene system as the SO_2-adduct, ozonolysis, and thermal cheletropic extrusion of SO_2 in the presence of Na_2CO_3.

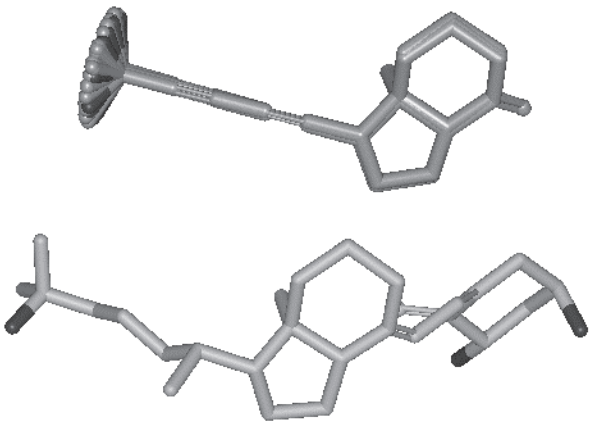


Fig. 1. Superposition of the modelled low energy conformers of analogue 15 (partial structure) (left); For comparison, the Moras conformation of calcitriol 1 (right)

Some of the key intermediates discussed above have been in particular used to prepare calcitriol analogues with modified side chains wherein the side chain hydroxyl oxygen atom is confined to particular regions of space relative to the rigid C/D-ring system. These were designed as probes to help identify the optimum agonistic or "active" conformation of the vitamin D hormone. One important result from our studies is the observation of high biological potency only in the 17*Z* isomer (14), derived from 9(i), of the two 17(20)-dehydro-calcitriols[8].

The crystal structure of a vitamin D receptor ligand binding domain (VDR-LBD) construct, with the bound ligand revealing the active side chain conformation in calcitriol[9], and in the LEO super-agonists 20-epi-calcitriol (MC1288) and KH1060 (ref.[10]), have been solved by the Moras group. The revelation that all three compounds anchor the *same* LBD amino-acid residues with the side chain hydroxyl, and furthermore have an identical location for the hydroxyl oxygen atoms relative to the seco-steroidal nucleus, provide a rational basis for selecting the optimal agonistic side chain architecture. We have designed an analogue (21-nor-calcitriol-20(22),23--diyne, 15) in which a very similar location of the side chain oxygen is actually built in rigidly. (The only residual flexibility in the side chain is due to the free rotation about the C-17:C-25 axis, but this gives rotamers (see Fig. 1) that have

equal energy in a vacuum.) Compound 15 was prepared from the aldehyde 10(i) *via* a Sonogashira cross coupling of the derived terminal alkyne with the requisite bromo-alkyne side chain building block.

REFERENCES

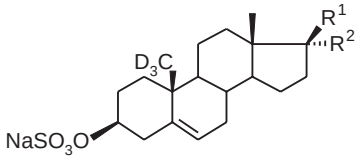
1. Calverley M. J.: Tetrahedron *43*, 4609 (1987).
2. Scheme: Information from LEO work on the established reaction sequences can be found in the proceedings of the triennial international workshops on Vitamin D: a,b: ref.[1,3–8]; c,d: ref.[3,4]; e: ref.[5]; f,g: ref.[6]; h: ref.[7]; j: ref.[8].
3. Bretting C., Calverley M. J., Binderup L., in: *Vitamin D* (Norman A. W., Bouillon R., Thomasset M., ed.), p. 159. Walter de Gruyter, Berlin 1991.
4. Calverley M. J., Binderup E., Binderup L., in: *Vitamin D* (Norman A. W., Bouillon R., Thomasset M., ed.), p. 163. Walter de Gruyter, Berlin 1991.
5. Calverley M. J., Pedersen H.: WO 98/18759 (1998).
6. Hansen K., Calverley M. J., Binderup L., in: *Vitamin D* (Norman A. W., Bouillon R., Thomasset M., ed.), p. 161. Walter de Gruyter, Berlin 1991.
7. von Daehne W., Mork Hansen C., Hansen D., Mathiasen I. S., in: *Vitamin D* (Norman A. W., Bouillon R., Thomasset M., ed.), p. 81. University of California Riverside 1997.
8. Calverley M. J., Bjorkling F., Blaehr L. K., Bretting C., Grue-Soerensen G., von Daehne W., in: *Vitamin D* (Norman A. W., Bouillon R., Thomasset M., ed.), p. 36. University of California Riverside 2000.
9. Rochel N., Wurtz J. M., Mitschler A., Klaholz B., Moras D.: Mol. Cell *5*, 173 (2000).
10. Tocchini-Valentini G., Rochel N., WurtzJ. M., Mitschler A., Moras D.: Proc. Natl. Acad. Sci. U.S.A. *98*, 5491 (2001).

SYNTHESES OF DEHYDROEPIANDROSTERONE AND PREGNENOLONE SULFATES LABELED WITH DEUTERIUM

IVAN ČERNÝ[a], MARIE BIČÍKOVÁ[b], MARTIN HILL[b], RICHARD HAMPL[b], MILOŠ BUDĚŠÍNSKÝ[a], VLADIMÍR POUZAR[a]

[a]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, [b]Institute of Endocrinology, Národní 8, Prague 1, Czech Republic
E-mail: cerny@uochb.cas.cz, rhampl@endo.cz

Labeling of steroids with stable isotopes has broad application in structural analysis and tracing in biological tissues. Use of deuterium in combination with gas chromatography – mass spectroscopy assay method requires the presence of at least two (better three) deuterium atoms substituting protium for attaining sufficient sensitivity. It is necessary to use such positions on the steroid skeleton, where deuterium is sufficiently stable toward protium exchange and where the steroid is not altered by metabolic processes under study. The aim of the project is a synthesis of two new deuterated derivatives,



1: $R^1 + R^2 = O$
2: $R^1 = COCH_3$, $R^2 = H$

dehydroepiandrosterone sulfate (DHEAS) and pregnenolone sulfate (PregS) with three deuterium atoms in position 19.

Corresponding 19-hydroxy derivatives of DHEA and pregnenolone, accessible by radical substitution of parent derivatives[1], were used as key intermediates. In both series, transformation into 6β-methoxy-3α,5α-cyclo derivatives changed steroid skeleton stericity and enabled successful oxidation into 19-oic acid. The cyclo derivative was cleaved and 3β-hydroxy-5-ene moiety was re-formed. The first two atoms of deuterium were introduced by lithium deuteride reduction of correspondingly protected methyl ester. Third atom substituted the 19-hydroxy group using two step procedure[2]: mesylation and then iodide substitution with simultaneous reduction with zinc in the presence of deuterium oxide. After the removal of protecting groups, sulfates 1 and 2 were prepared by the sulfur trioxide – pyridine complex method.

Prepared labeled DHEAS and PregS will be used in the collaborating institute for a study of steroid metabolism in tumor cells from the defined structures of human brain. The results will contribute to better knowledge of brain neoplasia processes and their connections with known mechanisms of interaction of neurosteroid sulfates with neural cells receptors.

Supported by grant No. 203/02/1440/A GA CR; a part of research project Z4 055 905 AS CR.

REFERENCES

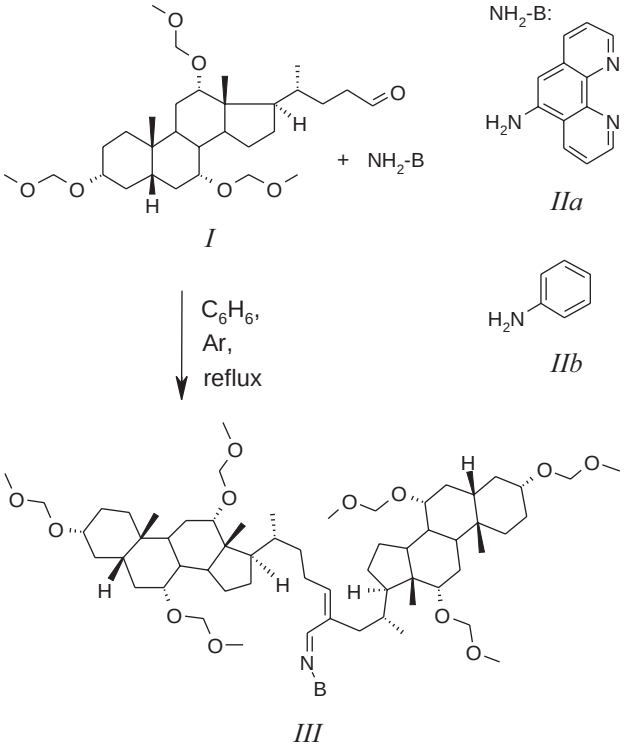
1. Kalvoda J., Heusler K., Ueberwasser H., Anner G., Wettstein A.: Helv. Chim. Acta *46*, 1361 (1963).
2. Kočovský P., Černý V.: Collect. Czech. Chem. Commun. *44*, 246 (1979).
3. Baxendale D., Kirk D. N., Rajagopalan M. S., Turner A. B.: J. Chem. Soc. C *1971*, 2564.

BASE CATALYSED CONDENSATION OF STEROIDAL ALDEHYDES

IVAN ČERNÝ[a], MYKHAYLO DUKH[a,c], LADISLAV KALVODA[b†], VLADIMÍR POUZAR[a], DAVID ŠAMAN[a], VLADIMÍR KRÁL[a,c], PAVEL DRAŠAR[a,c*]

[a]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, [b]Synlab, 250 97 Prague-Běchovice, [c]Institute of Chemical Technology, 166 28 Prague 6, Czech Republic
E-mail: drasar@uochb.cas.cz

The quest for modified receptors and self-assembly blocks[1,2] yielded into the synthesis of several unexpected condensation



products[3] resulting from the steroid side chain terminal aldehyde auto-condensation induced by basic catalysis.[4] The influence of different bases was studied.

Thus, (3α,7α,12α)-3,7,12-tris(methoxymethoxy)-5β-cholan-24-al (*I*, ref.[5]) was, upon basic catalysis condensed to branched dimeric condensation product with terminal aldehyde, which was further condensed without isolation with 5-amino-1,10-phenanthroline (*IIa*) in benzene. This reaction was compared with reactions of some other amines, and with aniline a similar condensation product was formed, whereas with pyridine and 1,10-phenanthroline there was no such condensation observed. After chromatographic purification, imines *III* were obtained in both positive cases, however, the condensation product with parent unchanged aldehyde *I* was not detected in any case.

The resulting compounds were reduced to the one, which could exhibit strong organogelating properties as demonstrated by Shinkai (cf. e.g.[6]), the studies on this phenomenon are being performed[7].

The support from the research project Z4 055 905 (MD, IC, VP), from the Ministry of education of the CR, project No. 223300006 (PD) as well as from Spechem Ltd., Prague is greatly acknowledged.

REFERENCES

1. (a) Dukh M., Drašar P., Černý I., Pouzar V., Shriver J. A., Král V. M., Sessler J. L.: Supramol. Chem. *14*, 237 (2002); (b) Dukh M., Černý I., Urbanský M., Pouzar V., Král V., Drašar P.: Czech 290491 (2002); (c) Dukh M., Šaman D., Kroulík J., Černý I., Pouzar V., Král V., Drašar P.: Tetrahedron *59*, 4069 (2003).
2. (a) Kochana J., Parczewski A.: Chem. Anal. *42*, 411 (1997); (b) Vanko G., Nagy S., Homonnay Z., Vertes A.: Hyperfine Interact. *126*, 163 (2000).
3. Šaman D., Dukh M., Drašar P., Král V., Kolehmainen E.: *2001 Nordic NMR Symposium, and 23rd Finnish NMR Symposium, Helsinki Finland*, Book of Abstracts, Yliopistopaino Helsinki, 2001, p. 55.
4. Dukh M., Kalvoda L., Šaman D., Černý I., Pouzar V., Král V. M., Drašar P.: Chem. Listy *96*, 400 (2002).
5. Dukh M., Černý I., Pouzar V., Král V., Drašar P.: Chem. Listy *95*, 749 (2001).
6. Jung J. H., Nakashima K., Shinkai S.: Nano Lett. *1*, 145 (2001).
7. Dukh M., Kalvoda L., Kroulík J., Šaman D., Pouzar V., Černý I., Král V. M., Drašar P.: Chem. Listy *96*, 956 (2002).

ECDYSTEROID BIOSYNTHESIS IN PLANTS

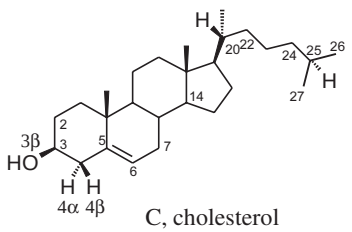
JOSEP COLL

Instituto de Investigaciones Químicas y Ambientales de Barcelona "Josep Pascual Vila", Consejo Superior de Investigaciones Científicas, Jordi Girona 18-26, 08034 Barcelona, Spain
E-mail: jctqob@iiqab.csic.es

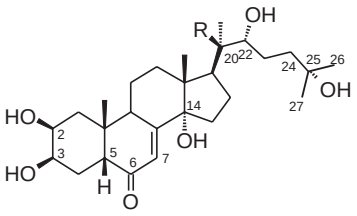
A summary of the efforts to disclose the biosynthetic pathway to ecdysteroids is presented in this overview. (*Abbreviations* – ECD, ecdysteroid(s), E, ecdysone; 20E, 20-hydroxyecdysone; 5,20E, polypodine B; 2dE, 2-deoxyecdysone; 25d20E = PoA, ponasterone A; 3D2,22,25dE, 3-dehydro--2,22,25-trideoxyecdysone; E3P, ecdysone 3-phosphate; C, cholesterol; L, lathosterol; MVA, mevalonic acid).

Isoprenoid biosynthesis involves the so-called mevalonic acid pathway (starting with acetate, activated as acetyl coenzyme A) or a mevalonate-independent pathway in the early steps[1]. Both lead to isopentenyldiphosphate (IPP) as the biological equivalent of isoprene. Higher isoprenoids are synthesized by condensation of IPP to the isomeric DMAPP or other prenyldiphosphates. Triterpene and sterol formation are intermediate steps towards ecdysteroid biosynthesis. Since ecdysone (E) is a pentahydroxycolestenone, conversion of cholesterol (C) or related compounds to 5β-diketol (14-hydroxycholest-7-en-3,6-dione, the last identified intermediate in the biosynthesis of E and 20E) has been defined as a "black box"[2].

Early studies were concerned with the finding of suitable precursors, labeled C being one in leaves of *Podocarpus elata* seedlings[3], whereas cholest-4-en-3-one, 25-hydroxyC, C-5α, 6α-epoxide and C-5β,6β-epoxide were not. C administration



C, cholesterol



E = ecdysone, R = H
20 E = 20-hydroxyecdysone, R = OH

to leaves of *Podocarpus macrophyllus* seedlings[4] resulted in formation of ponasterone A and 20E.

In *Polypodium vulgare*, the incorporation of [2-^{14}C]-MVA into 20E, and of [4-^{14}C]-C into E, 20E, and 5,20E was demonstrated[5], but in fact, cholest-7-en-3β-ol was more efficiently incorporated[6], a result pointing out the possible intermediacy of 7-dehydroC, as for insects. Stereospecific elimination of 7β-H was determined using doubly labeled cholesterol (4-^{14}C; 7α or 7β-$^{3}H_1$) in *P. vulgare* young shoots and *Taxus baccata*[7]. The fates of 3α-,4α- and 4β-H atoms during formation of A/B ring junction was also investigated, the label being retained but migration of ^{3}H from 3α to C-4 and 4β to C-5 occurred[8]. The disclosure of the presence of other ECD in *P. vulgare*, namely 24E, 20,24E, 5,20,24E, 24PoA and 26PoA, pointed out a more complex biosynthetic pathway[9].

According to the function present at C-25 two major structural types may be considered: 25-hydroxy-ECD and 25-deoxy-ECD. For 25-hydroxy-ECD the plausible intermediate precursor is E. Biotransformation of [23,24-^{3}H]-E, by prothalli resulted in 38 % labeled 20E and 3 % 5,20E after 1 week, but after 3 weeks the level reached 61 % 20E, and 20,24E was present at a 3 % rate (24E was not detected: either the biosynthetic path involves 20E, or 24E formed first is rapidly or preferably converted). After 3 weeks, all detected ECD from prothalli derived from a species of higher production of 24 hydroxylated ECD, were labeled including 24E (2 %). The relatively low amount of labeled 20,24E with respect to control, suggested a biosynthesis involving precursors other than 20E or 24E. As expected, the 25-deoxy-ECD were not labeled. From [2-^{14}C]-MVA, labeled 26PoA was formed at the same rate as the unlabeled compound, whereas 24PoA amount was higher than control after 1 week, but became similar after 3 weeks. PoA was tentatively identified in very low amount (1 %). [4-^{14}C]-C incorporation was observed in all reported ECD, with a similar profile than observed for MVA, but the relative proportion of E was lower. This result suggests that the authentic C27 sterol biosynthetic precursor from MVA is not cholesterol itself[10,11].

22*S*-Cholesterol is not converted into E by *P. vulgare* prothalli or calli, whereas 22*R*-C is, and also 20E is formed from E or PoA (25d20E). Different ECD, not occurring in *P. vulgare,* are formed from 3D2,22,25dE incubation. 3D2,22,25dE may be oxidized to 3D2,22dE (by a 25-OHase) or reduced (3α--or 3β-reductase) to 2,22,25d3αE (by both tissues) or 2,22,25dE (in calli). 2,22,25d3αE is converted into 2,22d3aE and an unidentified compound in prothalli. 2,22,25dE may be oxidized to 22,25dE or 2,22dE. 2,22dE may also proceed from 3D2,22dE, and yield 22dE in turn[12].

In spinach (possessing exclusively 7-ene sterols), the evidence suggests formation of lathosterol (L) from MVA, but biosynthesis of L is apparently coordinately regulated with the levels of formed E, E3P and 20E at the final stages[13]. Labeled E yields E3P and 20E, and E3P yields 20E. Formation of E or 20E polyphosphates (EpolyP, 20EpolyP) exerts a negative feedback in ecdysteroid biosynthesis.

In *Serratula tinctoria* sterol contents analysis evidenced a strong accumulation of triterpenes as amyrins (4,4-dimethylsterols). The sterol profile in roots consisted of sitosterol, stigmasterol, campesterol and cholesterol as main components, and sitostanol, stigmastanol, 24-methylene-cholesterol, lathosterol and desmosterol as minor ones[14]. The main ECD present are 20E and 20E3A, and as a minor one 5,20E. In whole plants, a more complex profile includes: 20E, 5,20E, 22D20E, 20E2A, 20E3A, 20E22A, 20E2,22A, 20E3,22A, 24PoA, Post, 3-epi-Post, Rub, 3-epi-Rub, 5Bub, and MaC[15]. Radiolabeled C was significantly incorporated into ecdysteroids (more than 0.1 % of the incorporated C as radioactive 20E). Minor ECD were presumably 5,20E and 20E3A (ref.[16]).

Several radiolabeled substrates were incorporated by *Zea mays* into ECD conjugates primarily (E and 20E) (ref.[17]). From MVA, lathosterol (present as a ca. 0.5 % of the total sterol composition) and cholesterol (present at 2.5 %) were endogenously biosynthesized, but specific activity for L was 3 to 6 times that of C. E was incorporated into EPP and EpolyP.

The ECD compounds isolated from *Ajuga* plants has been reviewed[18]. It is remarkable the presence of mixtures of carbon skeletons of 27, 28 or 29 units, pointing out possible differences in biosynthetic pathways. The biosynthesis of ecdysteroids (and sterols) in *Ajuga reptans* var. *atropurpurea* hairy roots has also been reviewed[19].

In contrast to the previously reported observations, the 3α-,4α- and 4β-hydrogen atoms in C were all retained at their original positions after conversion to 20E, and the origin of the 5β-hydrogen in 20E was found to be C-6 (70 %; the remaining 30 % from other source). Labeled lathosterol was fed and the resulting 20E established unambiguously the loss of 6α-H and migration of 6β-H to C-5, supporting 7DC as the required intermediate. Thus, the pathway C → 7DC → 7DC 5α,6α-epoxide → 5β-ketol → 5β-ketodiol →→ was proposed on the basis of conversion to 20E of the underlined intermediates. The conversion of 3β-hydroxy- and 2β,3β-hydroxy--5β-cholestan-6-one into 20E (ref.[20]) suggests the possibility of introduction of a double bond at the 7-position at a late stage (not necessarily prior to a carbonyl group at C-6). 5α-Isomer conversion to 20E (ketol or 7-en-ketol) was negligible, ruling out any involvement in biosynthesis.

Model compounds allowed to assign C-26 and C-27, respectively, as the pro-*S* and pro-*R* methyl resonances in 20E, and also the pro-*R* (C-26), pro-*S* (C-27) and C-25 in C. It was established that C-25 and C-27 proceed from an intact acetate unit (3,3[1] in MVA as implied from doubly labeled acetate feeding) and the pro-*R* C-26 is derived from C-2 of MVA, the reduction of the precursor 24-ene sterol taking place stereospecifically from the 25-*Si* face. As for 20E, C-25 hydroxylation was shown to occur mostly with retention, but inversion took place to a certain extent.

Clerosterol and 22Dclerosterol isolation from *A. reptans* was reported[21] and also the presence of both and C in the sterol fraction biosynthesized by *A. reptans* var. *atropurpurea*[22]. As

a likely intermediate, desmosterol was efficiently converted into clerosterol and to a small amount of codisterol, but less efficiently to C. On the other hand, 24-methyleneC was converted into clerosterol, 22Dclerosterol, and to a minor extent to campesterol.

Feeding studies recently reported[23], allowed to establish clerosterol as a precursor for Cy and isoCy, and evidence for its conversion into 29-norCy. Codisterol, as expected, was incorporated into 29-norCy but not into Cy or isoCy.

Work at CSIC has been financially supported by CICYT grants (BIO92/0398, PB94/0083, PB97/1253, AGL2001/2285) and predoctoral fellowships (CIRIT-GenCat to NR; MEC to JIS).

REFERENCES

1. Rohmer M.: Nat. Prod. Rep. *16*, 565 (1999).
2. Lafont R.: Arch. Insect Biochem. Physiol. *35*, 3(1997).
3. (a) Joly R. A., Svahn C. M., Bennett R. D., Heftmann E.: Phytochemistry *8*, 1917 (1969); (b) Sauer H. H., Bennett R. D., Heftmann E.: Phytochemistry *7*, 2027 (1968).
4. Hikino H., Kohama T., Takemoto T.: Phytochemistry *9*, 367 (1970).
5. de Souza N. J., Ghisalberti E. L., Rees H. H., Goodwin T. W.: Phytochemistry *9*, 1247 (1970).
6. Boid R.: *PhD Thesis*. University of Liverpool, Liverpool 1975; ref.[8]
7. Cook I. F., Lloyd-Jones J. G., Rees H. H., Goodwin T. W.: Biochem. J. *136*, 135 (1973).
8. Davies T. G., Lockley W. J. S., Boid R., Rees H. H., Goodwin T. W.: Biochem. J. *190*, 537 (1980).
9. Coll J., Reixach N., Sánchez-Baeza F., Casas J., Camps F.: Tetrahedron *50*, 7247 (1994).
10. Reixach N., Irurre-Santilari J., Casas J., Melé E., Messeguer J., Camps F.: Phytochemistry *43*, 597 (1996).
11. Messeguer J., Melé E., Reixach N., Irurre-Santilari J., Casas J.: *Biotechnology in Agriculture and Forestry*, Vol. 41., *Medicinal and Aromatic Plants X* (Bajaj Y. P. S., ed.), p. 333. 1998.
12. Reixach N., Lafont R., Camps F., Casas J.: Eur. J. Chem. *266*, 608 (1999).
13. Adler J. H., Grebenok R. J.: Lipids *30*, 257 (1995).
14. Corio-Costet M-F., Chapuis L., Mouillet J. F., Delbecque J-P.: Insect Biochem. Mol. Biol. *23*, 175 (1993).
15. Rudel D., Bathori M., Gharbi J., Girault J.P., Racz I., Melis K., Szendrei K., Lafont R.: Planta Med. *58*, 358 (1992).
16. Corio-Costet M.-F., Chapuis L., Delbecque J.-P.: *Biotechnology in Agriculture and Forestry*, Vol. 37, *Medicinal and Aromatic Plants IX* (Bajaj Y. P. S., ed.), p. 384. 1996.
17. Devarenne T. P., Sen-Michael B., Adler J. H.: Phytochemistry *40*, 1125 (1995).
18. Coll Toledano J.: Russ. J. Plant Physiol. *45*, 365 (1998).
19. Fujimoto Y., Ohyama K., Nomura K., Hyodo R., Takahashi K., Yamada J., Morisaki M.: Lipids *35*, 279 (2000).
20. Hyodo R., Fujimoto Y.: Phytochemistry *53*, 733 (2000).
21. Camps F., Coll J., Cortel A.: An. Quim. *79*, 228 (1983).
22. Yagi T., Morisaki M., Kushiro T., Yoshida H., Fujimoto Y.: Phytochemistry *41*, 1057 (1996).
23. Okuzumi K., Hara N., Fujimoto Y., Yamada J., Nakamura A., Takahashi K., Morisaki M.: Tetrahedron Lett. *44*, 323 (2003).

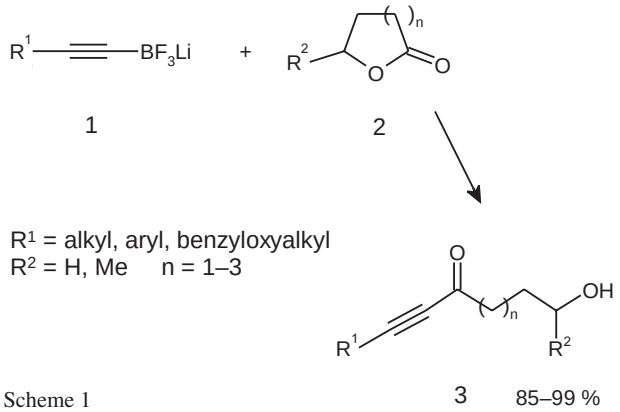
A NEW ROUTE TO FUNCTIONALIZED α-ALKYNONES AND THEIR USE IN SPIROACETAL SYNTHESIS

JAN DOUBSKÝ, BOHUMÍR KOUTEK

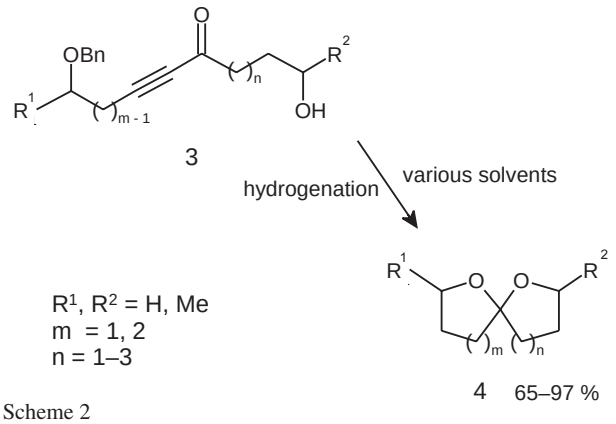
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: doubsky@uochb.cas.cz

The inherent ability of α-alkynones to undergo nucleophilic additions and cyclizations makes these compounds very useful intermediates in the synthesis of a wide range of more elaborated targets such as heterocycles, pheromones, drugs, and other naturally, as well as non-naturally derived bioactive compounds[1]. Considerable efforts have thus been devoted to the preparation of these conjugated ynones based on a variety of synthetic approaches, including especially: (*a*) the reaction of metalated terminal alkynes with acylchlorides, anhydrides, Weinreb amides, or lactones[2], (*b*) transition metal catalyzed cross-coupling reaction of 1-alkynes and alkynylstananes with acylchlorides[3], and (*c*) oxidation of corresponding α-alkynols. However, many of these protocols suffer from serious limitations, the most important being the use of relatively expensive catalysts and/or lack of versatility to accommodate a variety of substituents.

Herein, we wish to present an efficient procedure for the preparation of hydroxy-substituted a-alkynones 3 involving the general ring-opening reaction of five- to seven-membered lactones 2 with alkynyltrifluoroborates 1 (Scheme 1)[4], readily generated *in situ* by the addition of $BF_3.OEt_2$ to alkynyllithiums. This novel, operationally simple and high yielding (85–99 %) reaction allows a convenient and rapid access to α-alkynones substituted with valuable functional groups. The high degree of functionality of compounds 3 should confer very interesting synthetic potentialities especially for the construction of other (even chiral) structures, found in bioactive natural products.



Scheme 1



Scheme 2

One of the synthetic applications based on this strategy is illustrated on the spiroacetal synthesis (Scheme 2). The advantage of such approach consists in the use of benzyl-protected α-alkynols as starting compounds, which allows direct one--step transformation of α-alkynones 3 to spiroacetals 4. Spiroacetals 4, which are widely distribute in nature[5], could be thus obtained in good to excellent over all yields using this methodology.

REFERENCES

1. (a) Arcadi A., Martinelli F., Rossi E.: Tetrahedron *55*, 13233 (1999); (b) Midland M. M., Nguyen N. H.: J. Org. Chem. *46*, 4108 (1981); (c) Yu S., Li N.-S., Kabalka G. W.: J. Org. Chem. *64*, 5822 (1999).
2. Toczko J. F., Roberts J. C.: J. Org. Chem. *67*, 5032 (2002).
3. Grotjahn D. B., Van S., Combs D., Lev D. A., Schneider C., Rideout M., Meyer C., Hernandez G., Mejorado L.: J. Org. Chem. *67*, 9200 (2002).
4. Doubský J., Streinz L., Lešetický L., Koutek B.: Synlett *2003*, 937.
5. (a) Perron F., Albizati K. F.: Chem. Rev. *89*, 1617 (1989); (b) Francke W., Kitching W.: Curr. Org. Chem. *4*, 411 (2000).

CONFORMATIONALLY CONSTRAINED NEUROACTIVE STEROIDS: GABA-A RECEPTOR MODULATION BY 13,24-CYCLO-18,21-DINORCHOLANE DERIVATIVES

DOUGLAS F. COVEY*, **XIN JIANG, BRADLEY D. MANION, ANN BENZ, ALEX S. EVERS, CHARLES F. ZORUMSKI, STEVEN MENNERICK**

Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri, 63110, USA

Allopregnanolone (1) and pregnanolone (2) are potent allosteric modulators of γ-aminobutyric acid type A (GABA--A) receptors[1] (Fig. 1). GABA-A receptors are ligand-gated ion channels that regulate most fast neuronal inhibition in the central nervous system. GABA-A receptors are located on the surface of neurons in various regions of the mammalian brain. Binding of GABA to these receptors results in opening of the ion channel and, typically, the influx of chloride ions into the cell. This chloride influx hyperpolarizes the neuron thereby diminishing its activity. GABA-A receptors are the molecular targets of drugs with clinical uses as anesthetics, sedative--hypnotics, anticonvulsants and anxiolytics[2].

At low concentrations (~100 nM), steroids 1 and 2 potentiate the actions of GABA at GABA-A receptors. At higher concentrations (1–10 μM), these steroids directly gate the ion channel in the absence of added GABA. Neither the location nor the number of binding sites underlying these positive modulatory actions of steroids on GABA-A receptor function has been established.

Previous structure-activity relationship (SAR) studies have established the general structural features required for positive modulation of GABA-A receptor function by steroids[3]. These features include: (*1*) a hydrogen bond donor group in the 3α-position; and (*2*) a hydrogen bond acceptor group in the 17β-position. Surprisingly, the stereochemistry of the A,B-ring fusion is generally of little importance. For example, the concentration-response relationships for the actions of steroids 1 and 2 at GABA-A receptors as well as their dose-response relationships for their anesthetic actions in rats are very similar[3–5].

Recently, conformationally constrained analogues (D-ring epoxides and oxetanes) of steroids 1 and 2 were studied to investigate further the optimal location of the D-ring hydrogen bond acceptor group. From that study, it was concluded that this group should be near perpendicular to the plane of the D-ring above C-17 (ref.[6]). We have prepared and studied a series of 13,24-cyclo-18,21-dinorcholane analogues (3–6) to search for alternate locations for the D-ring hydrogen bond acceptor group (Fig. 2). The cyclo ring of these analogues

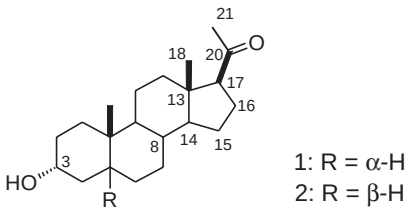
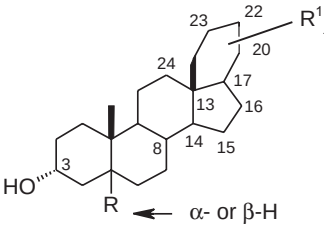


Fig. 1.



3: R^1 = 20-oxo or other H-bond acceptor
4: R^1 = 22-oxo or other H-bond acceptor
5: R^1 = 23-oxo or other H-bond acceptor
6: R^1 = 24-oxo or other H-bond acceptor

Fig. 2.

provides an invariant framework upon which hydrogen bond acceptor groups can be positioned to search for receptor hydrogen bond interactions across the entire β-face of the steroid D-ring.

Cyclosteroids 3 (R^1 = oxo) are prepared from either steroid 1 or 2 by a reaction sequence that involves the introduction of a nitrile group on C-18, reduction of the nitrile group to an aldehyde group and then its cyclization with the 17β-acetyl group to give a 13,24-cyclo-18,21-dinorchol-22-en-20-one product[7–9]. The remaining cyclosteroids 4–6 are prepared from these cyclization products by chemical procedures that in essence reposition the 20-oxo group at the remaining positions on the cyclo ring. The cyclosteroids were examined for their ability to: (*1*) non-competitively displace a radioligand bound at the picrotoxin binding site of GABA-A receptors (TBPS--binding); (*2*) affect either GABA-mediated (potentiation) or direct gating of chloride currents at rat α1β2γ2 type GABA-A receptors expressed in *Xenopus laevis* oocytes and (3) cause loss of either the righting or swimming response of *Xenopus laevis* tadpoles.

The SARs for the cyclosteroid analogues (R_1 = oxo) of steroids 1 and 2 are closely similar suggesting that the binding sites for both 5α- and 5β-reduced steroids recognize the steroid D-ring in a similar manner. The order of potency for displacement of [^{35}S]-TBPS binding was: 1≈2>6≥5>3>4. Similar relative orders of potencies were also found in the other two bioassays. Introduction of a Δ^{22} double bond into cyclosteroids 6 yields analogues with activities comparable to those of steroids 1 and 2.

The most surprising results from the study were those obtained with the 24-oxocyclosteroids 6. The 24-oxo group in these compounds is located over the C-ring and projects toward C-8. The 20-oxo group of steroids 1 and 2, regardless of its three-dimensional orientation in the freely rotating acetyl group, is never oriented toward C-8. Hence, it is difficult to envision how the 24-oxo group of cyclosteroids 6 and the 20-oxo group of steroids 1 and 2 could interact with the same hydrogen bond donor group on the receptor. It seems more likely that there are multiple receptor hydrogen bond donor groups arranged in a band over the steroid D-ring that have the potential to interact with hydrogen bond acceptor groups located in different places on or near the D-ring. Ongoing studies with cyclosteroids having different types of hydrogen bond acceptor groups at each position of the cyclo ring along with studies of cyclosteroid derivatives in which the cyclo ring is a five-membered ring (18,21-cyclopregnanes) will further refine the location of the receptor hydrogen bond donor groups.

REFERENCES

1. Lambert J. J., Belelli D., Harney S. C., Peters J. A., Frenguelli B. G.: Brain Res. Rev. *37*, 68 (2001).
2. Zorumski C. F., Mennerick S., Isenberg K. E., Covey D. F.: Curr. Opin. Invest. Drugs *1*, 360 (2000).
3. Phillipps G. H.: J. Steroid Biochem. *6*, 607 (1975).
4. Gyermek L., Iriarte J., Crabbé P.: J. Med. Chem. *11*, 117 (1968).
5. Covey D. F., Evers A. S., Mennerick S., Zorumski C. F., Purdy R. H.: Brain Res. Rev. *37*, 91 (2001).
6. Anderson A., Boyd A. C., Clark J. K., Fielding L., Gemmell D. K., Hamilton N. H., Maidment M. S., May V., McGuire R., McPhail P., Sansbury F. H., Sundaram H., Taylor R.: J. Med. Chem. *43*, 4118 (2000).
7. Mičková R., Hutlová H., Pihera P., Protiva J., Schwarz V.: Collect. Czech. Chem. Commun. *50*, 1110 (1985).
8. Kalvoda J., Meystre Ch., Anner G.: Helv. Chim. Acta *49*, 424 (1966).
9. Perard S., Viger A., Marquet A.: Steroids *55*, 271 (1990).

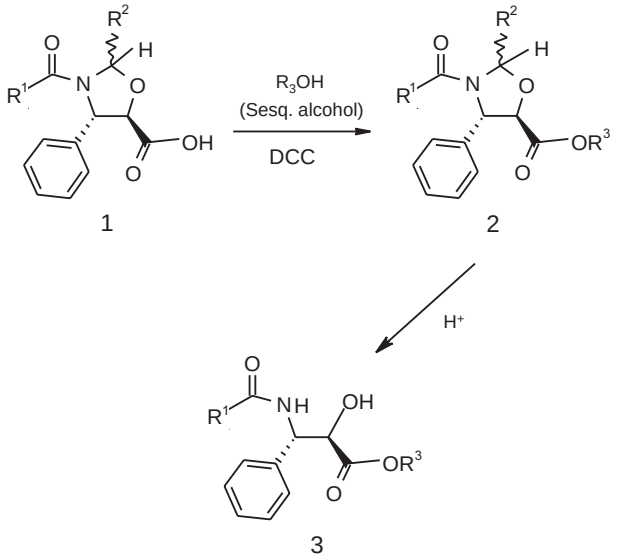
SYNTHESIS AND CYTOTOXIC PROPERTIES OF *N*-ACYLPHENYLISOSERINATES OF SESQUITERPENOID ALCOHOLS OF *LACTARIUS* ORIGIN

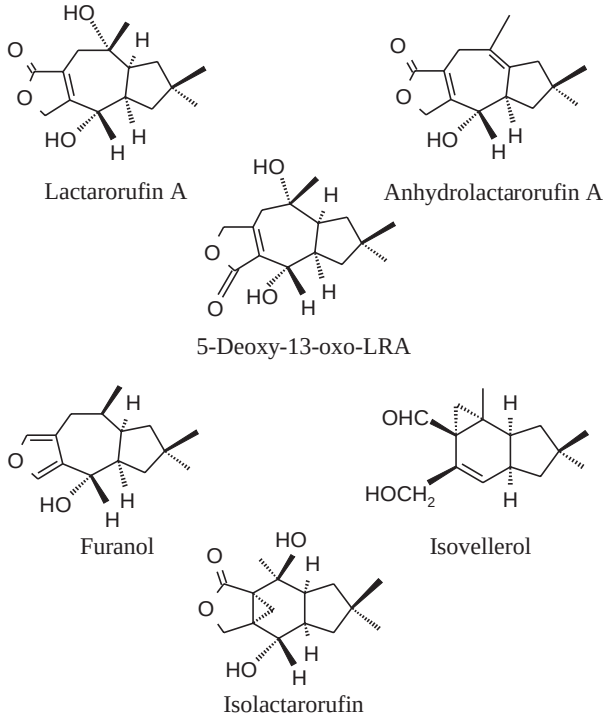
RAFAŁ BARYCKI[a], PIOTR KOPCZACKI[a], MARIA GUMUŁKA[a], MAREK MASNYK[a], AGATA SAROSIEK[a], WŁODZIMIERZ DANIEWSKI[a], MIROSŁAW KOBUS[b], MIROSŁAW ŁUCZAK[b]

[a]Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44, 01-224 Warsaw, [b]Department of Medical Microbiology, Medical University of Warsaw, Chałubińskiego 5, 02-004 Warsaw, Poland

Important biological properties of Taxol® i.e. 13-*N*-benzoyl phenylisoserinate (2*R*,3*S*) of baccatin 3 prompted us to synthesize and to check cytotoxic properties of various *N*-acyl--(2*R*,3*S*)-phenylisoserinates of several sesquiterpenoid alcohols of *Lactarius* origin. Suitably protected *N*-acylphenylisoserine (1) when reacted with sesquiterpenoic alcohols in presence of DCC gave appropriate esters (2). These, when hydrolyzed in acidic conditions produced *N*-acylphenylisoserinates (3).

The compounds are sesquiterpenoid analogues of Taxol® and Taxotere®. Some of them exhibited cytotoxic properties when tested on various human cancer cells, these are inve-





The typical examples of sesquiterpenoic alcohols

stigated. Structure cytotoxic activity relationship will be presented.

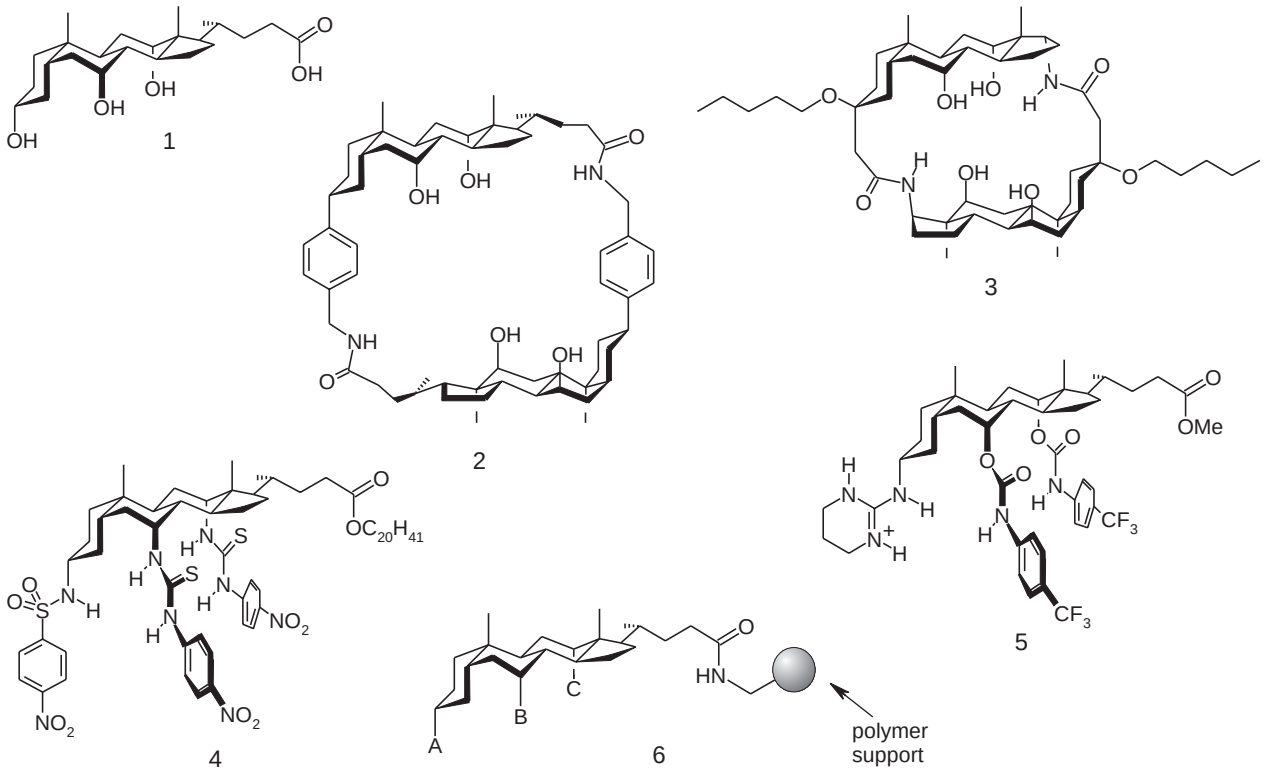
STEROIDAL SCAFFOLDS FOR BIOMIMETIC AND SUPRAMOLECULAR CHEMISTRY

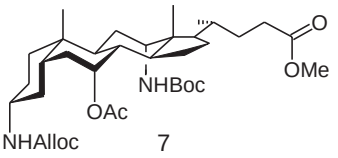
ANTHONY P. DAVIS

School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, U.K.
E-mail: Anthony.Davis@bristol.ac.uk

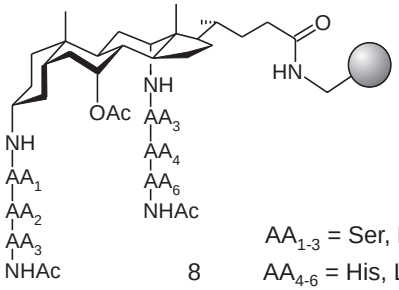
The steroid nucleus has proved valuable as an organising scaffold in supramolecular chemistry. It is rigid, extended, chiral, and available in various incarnations from natural sources. Cholic acid 1 is especially useful, being inexpensive and possessing a nicely-spaced array of differentiable functional groups[1]. From this starting point it is possible to design systems with well-defined binding sites, bordered by various combinations of polar functional groups. These molecules may be used for studies in molecular recognition, and also have potential for biomimetic catalysis.

Over the past ~15 years, we have used 1 to construct a range of synthetic receptors. Early work focussed on carbohydrate recognition by macrocyclic "cholaphanes" such as 2 (ref.[2]). More recently we have turned to anionic substrates, preparing a series of electroneutral, lipophilic receptors for inorganic anions, and also addressing the enantioselective recognition of carboxylates[3]. The former programme is illustrated by "cryptand" 3 and "cholapod" 4. The most recent receptors, exemplified by 4, are highly potent while maintaining solubility in non-polar media such as chloroform[4]. Exceptional binding constants result, measurable only through adaptation of an extraction method originally devised by Cram. This combination of affinity and lipophilicity has allowed the





7



8

AA_{1-3} = Ser, His, Gly

AA_{4-6} = His, Leu, Ala

exploitation of cholapods as synthetic "flippases"[5] and suggests applications in membrane transport, ion selective electrodes etc.

The enantioselective carboxylate receptors are typified by guanidinium cation 5. This species has been shown to extract several *N*-acetyl amino acids from water into chloroform with encouraging enantioselectivities (ca. 80 % e.e.)[6]. In transport experiments, a lipophilic variant has shown similar selectivities, confirming the prospect for "catalytic" applications of such receptors in separation systems[7]. Electroneutral analogues are also under study; these are more readily synthesized and convenient to handle, while retaining useful levels of selectivity[8].

Finally, the bile acid framework is a useful tool for combinatorial chemistry[9]. Conversion of 1 into differentially-protected amino-steroids[10] allows ready access to libraries of "receptor-like" structures 6. These may be screened for recognition properties, and have potential for enzyme-like catalysis. For example, the intermediate 7 has been prepared in good yield[11] and used to make a library of general form 8 (ref.[12]). Screening identified members with unusual susceptibility to acylation, perhaps pointing the way to a "synthetic hydrolase".

REFERENCES

1. Davis A. P., Bonar-Law R. P., Sanders J. K. M., in: *Comprehensive Supramolecular Chemistry*, Vol. 4, *Supramolecular Reactivity and Transport: Bioorganic Systems* (Murakami Y., ed.), pp. 257–286. Pergamon, 1996.
2. Davis A. P., Wareham R. S.: Angew. Chem., Int. Ed. Engl. *38*, 2978 (1999).
3. Davis A. P., Joos J.-B.: Coord. Chem. Rev. *240*, 143 (2003).
4. Ayling A. J., Pérez-Payán M. N., Davis A. P.: J. Am. Chem. Soc. *123*, 12716 (2001).
5. Lambert T. N., Boon J. M., Smith B.D., Pérez-Payán M. N., Davis A. P.: J. Am. Chem. Soc. *124*, 5276 (2002).
6. (a) Davis A. P., Lawless L. J.: Chem. Commun. *1999*, 9; (b) Lawless L. J., Blackburn A. G., Ayling A. J., Pérez-Payán M. N., Davis A. P.: J. Chem. Soc., Perkin Trans. 1 *2001*, 1329.
7. Baragaña B., Blackburn A. G., Breccia P., Davis A. P., de Mendoza J., Padron-Carrillo J. M., Prados P., Riedner J., de Vries J. G.: Chem. Eur. J. *8*, 2931 (2002).
8. Siracusa L., Hurley F. M., Dresen S., Lawless L. J., Pérez-Payán M. N., Davis A. P.: Org. Lett. *4*, 4639 (2002).
9. (a) Boyce R., Li G., Nestler H. P., Suenaga T., Still W. C.: J. Am. Chem. Soc. *116*, 7955 (1994); (b) Cheng Y. A., Suenaga T., Still W. C.: J. Am. Chem. Soc. *118*, 1813 (1996).
10. Kasal A., Kohout L., Lebl M.: Collect. Czech. Chem. Commun. *60*, 2147 (1995).
11. Barry J. F., Davis A. P., Pérez-Payán M. N., Elsegood M. R. J., Jackson R. F. W., Gennari C., Piarulli U., Gude M.: Tetrahedron Lett. *40*, 2849 (1999).
12. De Muynck H., Madder A., Farcy N., De Clercq P. J., Pérez-Payán M. N., Öhberg L. M., Davis A. P.: Angew. Chem., Int. Ed. Engl. *39*, 145 (2000).

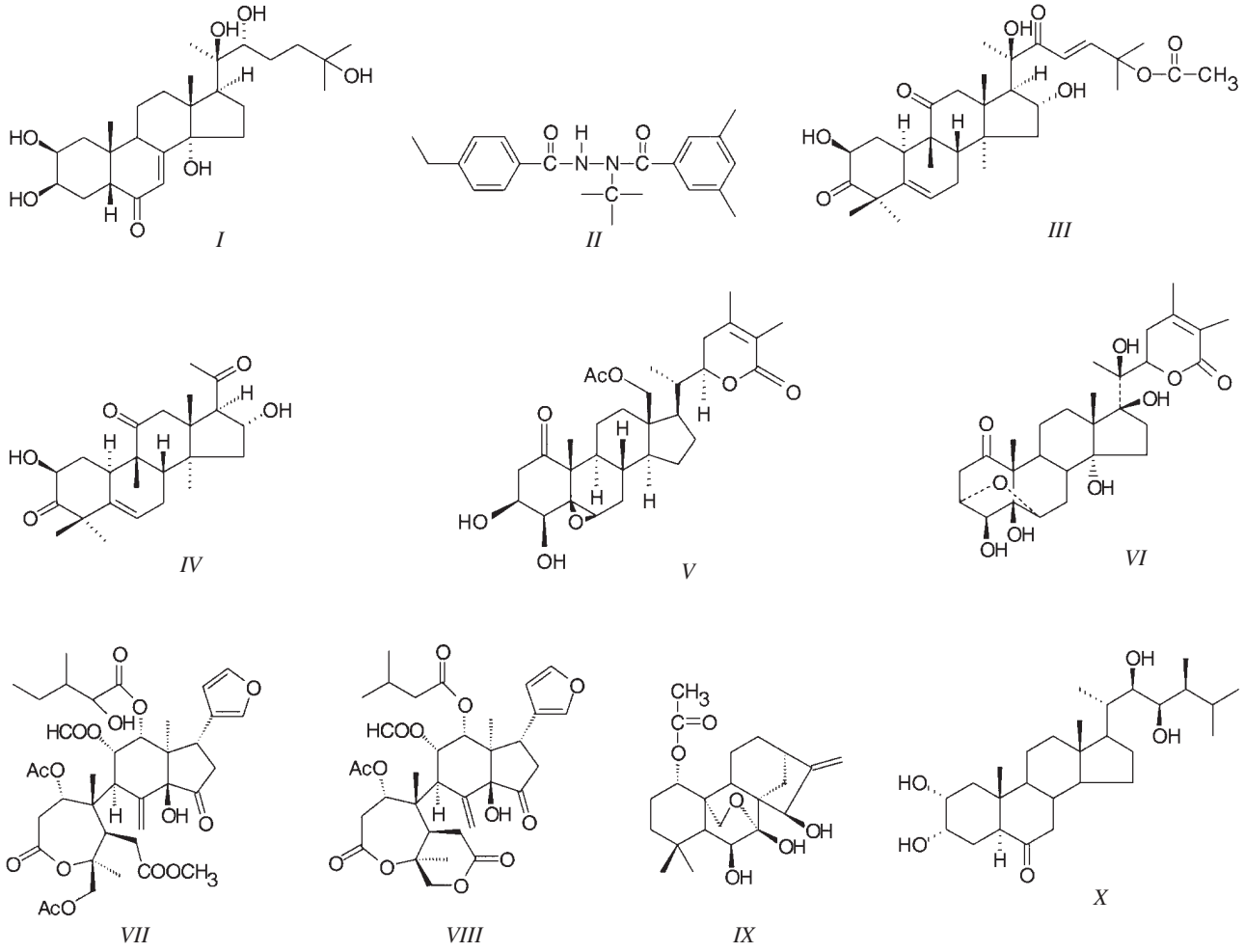
ISOPRENOIDS AS ECDYSTEROID RECEPTOR AGONISTS AND ANTAGONISTS

LAURENCE DINAN*

Department of Biological Sciences, University of Exeter, Hatherly Laboratories, Prince of Wales Road, Exeter, Devon, EX4 4PS, U.K.
E-mail: L.N.Dinan@ex.ac.uk

Ecdysteroids are the steroid hormones of arthropods and probably of other invertebrate Phyla too[1]. In insects, where their functions have been most extensively studied, they have been implicated in the regulation of moulting, metamorphosis, reproduction, embryonic development and diapause. In common with vertebrate steroid hormones, the majority of the actions of ecdysteroids are mediated by intracellular receptors, which are members of the nuclear receptor superfamily[2]. In the case of arthropod ecdysteroid receptors, the receptor complex is a heterodimer consisting of the ecdysteroid receptor (EcR) protein and the Ultraspiracle (USP) protein, which is a homologue of retinoid X receptor (RXR). Although the ecdysteroid binds to EcR, EcR must be bound to USP (or RXR) for high affinity binding of the ligand[3]. There is currently no definitive ligand for USP, although it has been suggested that juvenile hormone may bind[4]. It is generally accepted that 20-hydroxyecdysone (20E; I) is the natural ligand for EcR under most circumstances. However, ca. 50 different ecdysteroids have been isolated from animals (zooecdysteroids), most of which are biosynthetic precursors, further metabolites or storage forms. Further, ecdysteroids also occur in certain plant species at much higher concentrations (up to 3 % of the dry weight in exceptional cases, but more typically 0.1–1 % (ref.[5])). These phytoecdysteroids are believed to reduce predation by non-adapted invertebrate predators, either as antifeedants or by hormonal disturbance after ingestion. The structural diversity of phytoecdysteroids is large, with ca. 250 analogues known, and encompassing most of the known zooecdysteroids[6].

Owing to the essential role of ecdysteroids during all



stages of development of insects, the ecdysteroid receptor complex is an attractive target for the development of new insect pest control agents. Unfortunately, the ecdysteroids themselves do not have the necessary chemical, environmental and economical attributes for exploitation. Thus, it is necessary to identify non-steroidal compounds acting as ecdysteroid agonists and antagonists. The first class of compounds identified was the diacylhydrazines, e.g. tebufenozide (RH-5992; II), which, along with two other analogues, has been commercialised as an insecticide[7]. These compounds interact with the ligand binding site of EcR as weak agonists. Since they are only slowly metabolised in insects, they can accumulate to concentrations adequate for the disruption of moulting and development.

Over the past decade we have pursued a strategy for the identification of natural products acting as ecdysteroid receptor agonists and antagonists and an understanding of their structure-activity relationships, with the ultimate goal of the design of simpler, target-specific insecticidal compounds. Central to this strategy was the development of a simple, robust, microplate-based bioassay for ecdysteroid agonists and antagonists, based on the ecdysteroid-responsive *Drosophila melanogaster* B_{II} permanent cell line[8]. This bioassay is appropriate for (*i*) the assessment of relatively crude plant extracts for ecdysteroid (ant)agonist activity, (*ii*) bioassay-guided fractionation of active extracts and (*iii*) the quantitative determination of the relative potencies of pure active compounds[9]. Extracts/compounds showing agonist activity almost certainly interact with the ligand-binding domain (LBD) of EcR. Antagonists are somewhat more complicated, since antagonism can be generated through actions at many sites in the ecdysteroid-regulated cascade. However, the B_{II} assay result can be further supported by use of cell-free radioligand receptor assays and gel-shift assays monitoring the interaction of the EcR/USP complexes with ecdysteroid response elements (EcRE) to determine if the compound interacts with the receptor or some other component of the signal transduction system. With this bioassay, we have (*i*) screened 5000 plant species and several hundred purified compounds for their activity and (*ii*) determined the potencies (EC_{50} values) of many analogues for QSAR/molecular modelling purposes. Amongst the active compounds are several classes of isoprenoids and this lecture will be concerned with the studies on these.

Ecdysteroids: The biological activities of ca. 150 purified ecdysteroid analogues have been determined. Almost all of these show agonist activity, with activities ranging over 6

orders of magnitude. These data have been used for molecular modelling (CoMFA[10] and 4D-QSAR[11]) and for the construction of a pharmacophore hypothesis for ecdysteroid binding to EcR. 4D-QSAR has further indicated the H-bonding capacities of the various O-containing functions around the molecule. The value of these models is that they generate testable hypotheses, which can be used to direct the synthesis of further analogues to assess and improve the models. Ultimately, these models can form the basis of a virtual screen to identify members of compound libraries which could interact with the EcR LBD, for example in the identification of potential endocrine disruptors of arthropods[12]. Only one ecdysteroid has been identified which possesses antagonistic activity in the B_{II} assay.

Cucurbitacins: A methanolic extract of seeds of *Iberis umbellata* (Cruciferae) showed antagonistic activity. Bioassay-guided fractionation of the extract identified cucurbitacins B (III) and D as the active compounds, active in the micromolar range against 20E at 5×10^{-8} M. Further studies revealed that cucurbitacins can displace radiolabelled ecdysteroid from the ecdysteroid receptor complex, antagonise 20E-induced stimulation of a transfected ecdysteroid-regulated reporter gene in *D. melanogaster* S2 cells and prevents the formation of 20E-induced formation of EcR/USP/EcRE complexes as examined by gel-shift assays. Initial QSAR studies indicated that the presence of an α,β-unsaturated ketone in the side-chain is associated with antagonistic activity. This is supported by the observations that hexanorcucurbitacin D (IV), which lacks the α,β-unsaturated ketone is agonistic and the side-chain analogue, 5-methylhex-3-en-2-one, is a weak antagonist[13].

Withanolides: A number of withanolides had been isolated from *Iochroma gesnerioides* (Solanaceae)[14] and these were assessed for their activity in the B_{II} bioassay[15]. Several of these, all with an α,β-unsaturated ketone in the side-chain (e.g. 2,3-dihydro-3β-hydroxywithacnistine; V), possess antagonistic activity. A range of other withastereroids has been assessed for activity, most of which were inactive. However, one (withaperuvin D; VI) was agonistic[16].

Limonoids: The antagonist activity of an extract of seeds of *Turraea obtusifolia* (Meliaceae) was found to be associated with prieurianin (VII) and rohitukin (VIII). The purified compounds possess only weak activities. Several other limonoids do not possess activity in the B_{II} assay. It is not yet clear whether the active limonoids interact with the EcR LBD[17].

Diterpenoid: The diterpenoid maocrystal E (IX) possesses agonist activity in the B_{II} bioassay and also displaces radiolabelled ecdysteroid from the EcR LBD[16].

Brassinosteroids: Owing to the chemical similarity between ecdysteroids and brassinosteroids (e.g. castasterone; X), it has been suggested that brassinosteroids might interact with ecdysteroid receptors and *vice versa*. A few publications provide evidence for weak agonist or antagonist activity of brassinosteroids in insect systems. However, this has not been supported by studies with the B_{II} assay[16]. Biochemically, the ecdysteroids and brassinosteroids are significantly different. A study has been performed to generate chemically brassinosteroid/ecdysteroid hybrids to identify which structural features confer biological activity in the respective bioassays[18].

Vertebrate steroids, sterols, bile acids, cardiac glycosides, saponins: Representatives of these classes do not possess ecdysteroid (ant)agonistic activity[16].

The rapid advance of computer-based QSAR methods is resulting in faster, more sophisticated modelling of complex data sets. These will permit more refined analysis of the B_{II} assay data for ecdysteroids. The cloning of EcR and USP genes from a wide range of arthropods permits their expression and analysis of ligand binding specificity of the active complex. This should identify Order-specific differences, which it might be possible to exploit in the development of new insecticides. Comparable models for the binding of ecdysteroid antagonists will provide further insights into ligand binding. The ecdysteroid receptor (or chimaeras of it) is being studied as a component of gene switch systems in mammalian or plant cells. While these systems do function, the potency of ecdysteroids is generally 100–100-fold less than in insect systems[19]. One possible explanation for this is altered conformation of the receptor complex in transfected cells. Examination of the binding specificity may identify strategies for the development of higher affinity ligands.

The biological activity of natural products as ecdysteroid (ant)agonists indicates possible roles for them in insect--plant relationships. Analysis of the distribution of ecdysteroid (ant)agonists in the plant world provides information about the chemotaxonomic value of these compounds[20] and the relationship to the geographical location of the species[21].

REFERENCES

1. Koolman J. (ed.): *Ecdysone*. Thieme Verlag, Stuttgart 1989.
2. Laudet V.: J. Mol. Endocrinol. *19*, 207 (1997).
3. Yao T. P., Forman B. M., Jiang Z. Y., Cherbas L., Chen J. D., McKeown M., Cherbas P., Evans R. M.: Nature *366*, 476 (1993).
4. Jones G., Jones D.: Insect Biochem. Mol. Biol. *30*, 671 (2000).
5. Dinan L.: Phytochemistry *57*, 325 (2001).
6. Lafont R., Harmatha J., Marion-Poll F., Dinan L., Wilson I. D.: *Ecdybase* (The Ecdysone Handbook, 3rd Edition) (2002); http://ecdybase.org, 4/6 2003.
7. Dhadialla T. S., Carlson G. R., Le D. P.: Ann. Rev. Entomol. *43*, 545 (1998).
8. Clément C. Y., Bradbrook D. A., Lafont R., Dinan L.: Insect Biochem. Mol. Biol. *23*, 187 (1993).
9. Dinan L.: Eur. J. Entomol. *92*, 271 (1995).
10. Dinan L., Hormann R. E., Fujimoto T.: J. Computer-aided Mol. Des. *13*, 185 (1999).
11. Ravi M., Hopfinger A. J., Hormann R. E., Dinan L.: J. Chem. Info. Comp. Sci. *41*, 1587 (2001).
12. Dinan L., Bourne P., Whiting P., Dhadialla T. S., Hutchinson T. H.: Environ. Toxicol. Chem. *20*, 2038 (2001).
13. Dinan L., Whiting P., Girault J.-P., Lafont R., Dhadialla T. S., Cress D. E., Mugat B., Lepesant J.-A.: Biochem. J. *327*, 643 (1997).
14. Alfonso D., Kapetanidis I.: Phytochemistry *36*, 179 (1994).
15. Dinan L., Whiting P., Alfonso D., Kapetanidis I.: Entomol. Exp. Appl. *80*, 415 (1996).
16. Dinan L., Bourne P. C., Meng Y., Sarker S. D., Tolentino R. B., Whiting P.: Cell. Mol. Life Sci. *58*, 321 (2001).

17. Sarker S. D., Savchenko T., Whiting P., Sik V., Dinan L. N.: Arch. Insect Biochem. Physiol. *35*, 211 (1997).
18. Voigt B., Whiting P., Dinan L.: Cell. Mol. Life Sci. *58*, 1133 (2001).
19. Lafont R., Dinan L.: *J. Insect Sci. 3:7* (2003); http: //www.insectscience.org/3.7.
20. Dinan L., Whiting P., Scott A. J.: Biochem. Syst. Ecol. *26*, 553 (1998).
21. Volodin V., Chadin I., Whiting P., Dinan L.: Biochem. Syst. Ecol. *30*, 525 (2002).

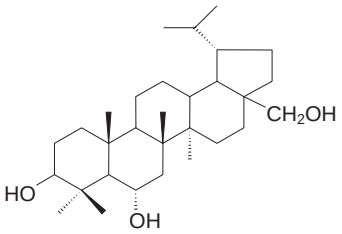
THE PREPARATION OF B-RING SUBSTITUTED LUPANE DERIVATIVES

MARTIN DRAČÍNSKÝ, JAN SEJBAL

Department of Organic Chemistry, Charles University, Albertov 6, 128 40 Prague 2, Czech Republic
E-mail: sejbal@natur.cuni.cz

New lupane type triterpenoids with 5(6) double bond were prepared from betulin using the method of partial demethylation on carbon C-4 and reconstruction of the ring A. A new method for selective oxidation of the secondary hydroxyl group in the presence of a primary hydroxyl group was studied during the reaction sequence leading to 5(6) unsaturated lupane derivative.

The hydroboration of the 5(6) double bond was studied and it was found to lead to 6α-hydroxy derivative. By the oxidation and following reduction of 6α-hydroxy derivative, various 6-oxo and 6β-hydroxy derivatives were prepared. The conformation of the ring A of new lupane type 3-oxo derivatives with a substituent or double bond on ring B was elucidated from ^{1}H NMR spectra (coupling constants and NOESY spectra) and molecular modelling (simulated annealing). The influence of B-ring substituents on the chemical shifts of protons of angular methyl groups was studied.



The comparison of lupane derivatives with the oxygen containing substituent in the position 6 prepared here with compounds isolated from various natural materials was performed. Seven structures formerly published as B-substituted lupane derivatives were found to be incorrect.

24-EPIBRASSINOLIDE AT SUBNANOMOLAR CONCENTRATIONS MODULATES GROWTH OF MOUSE HYBRIDOMA CELLS

F. FRANĚK[a], L. KOHOUT[b], T. ECKSCHLAGER[c]

[a]Institute of Experimental Botany, [b]Institute of Organic Chemistry and Biochemistry, Academy of Science of the Czech Republic, Prague, [c]Depertment of Pediatric Oncology, Charles University, Prague, Czech Republic

Brassinolides are known to stimulate plant growth and to posses antistress activities in plants. This work was aimed at exploring possible beneficial effects of 24-epibrassinolide on cultured mammalian cells. A mouse hybridoma, i.e. a hybrid lymphocyte cell line, was cultured either in standard serum-free medium, or in diluted medium in which the cell grew under nutritional stress. Steady-state parameters of semicontinuous cultures conducted at 24-epibrassinolide concentrations from 10^{-16} M to 10^{-9} M were evaluated. Typical effects of the agent that were observed both in standard and in diluted media were suppression of intracellular antibody level, increased value of mitochondrial membrane potential, higher fraction of cells in the G_0/G_1 phase and lower fraction of cells in the S phase. Alleviation of nutritional stress was observed in cultures conducted in diluted media. Viable cell density was significantly higher at 24-epibrassinolide concentrations 10^{-13} and 10^{-12} M. Results of this exploratory study show that the plant hormone 24-epibrassinolide may induce alterations of the cell division mechanism, of the energetic metabolism, and of secreted protein synthesis in a mammalian cell line.

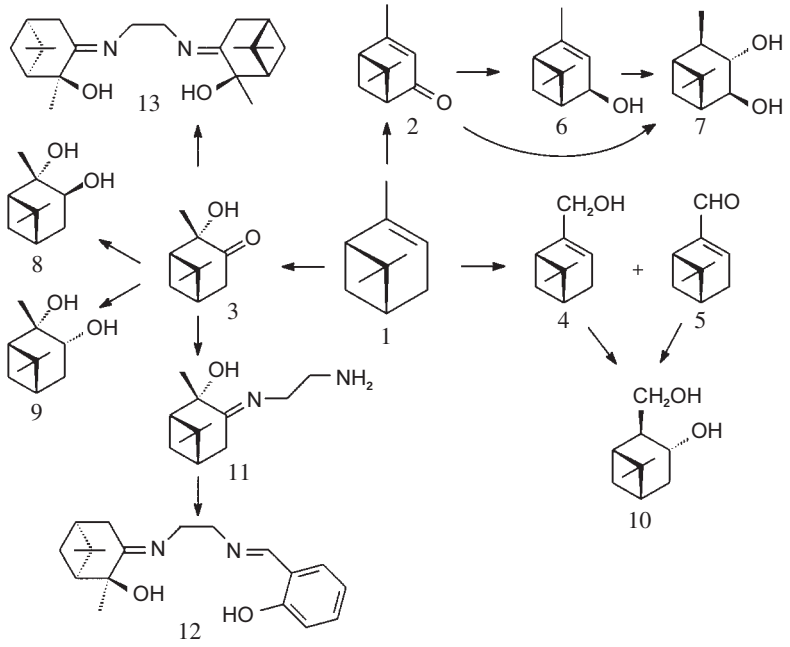
This work was supported by projects Nos. Z 4055905, 203/01/0083 and IBS4055304.

SYNTHESIS OF CHIRAL POLYFUNCTIONAL OXYGEN- AND NITROGEN-BEARING LIGANDS ON THE BASIS OF α-PINENE

LARISA L. FROLOVA, IRINA A. DVORNIKOVA, ALEXANDER V. KUTCHIN

Institute of Chemistry, Komi Science Center, Ural Division of the Russian Academy of Sciences, 48, Str. Pervomaiskaya, 167982, Syktyvkar, Russian Federation
E-mail: rubtsova-sa@chemi.komisc.ru

Optically active monoterpens of pinane, bornane and carane structure are the most available and cheap raw materials in chemistry of plant substances. O,N,S,P-bearing derivatives, obtained on this basis, are widely used as ligands for synthesis of chiral reagents and catalysts for asymmetrical synthesis becomes more important tool in organic chemistry. This paper deals with synthesis of optically active diols, amino- and imino-derivatives of α-pinene (1). Functionalization of (1) by such oxidants as O_2, $KMnO_4$, SeO_2 leads to the formation of verbenone (2), 2α-hydroxypinanone-3 (3), myrtenol (4) and mirtenal (5), respectively. A series of vicinal and 1,3-diols – *trans*-3,4-pinanediol (7), *cis*- (8) and *trans*-2,3-pinanediol (9),



3,10-pinanediol (10) was synthesized on the basis of these compounds with the use of hydride reduction reactions and hydroboration followed by oxidation. Mono- (11) and disubstituted symmetrical (13) and asymmetrical imines (12) are synthesized on the basis of ketol (3).

SYNTHESIS AND BIOACTIVITY OF BRASSINOSTEROIDS ANALOGUES WITH DIFFERENT FUNCTIONAL GROUPS AT C-6

LYDIA R. GALAGOVSKY[a], JAVIER A. RAMÍREZ[a], CARME BROSA[b], EMMA TERRICABRAS[b], ANNA MODOLELL[b]

[a]Department of Organic Chemistry, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 1428, Buenos Aires, Argentina. [b]Department of Organic Chemistry, C.E.T.S. Institut Químic de Sarriá, Universitat, Ramón Llull, Vía Augusta 390, 08017 Barcelona, Spain
E-mail: lyrgala@qo.fcen.uba.ar, brosa@iqs.url.es

Organofluorine compounds have recently attracted considerable attention in the fields of agrochemistry, pharmaceutical and material science[1–3].

In view of their unique biological properties, fluorinated steroids have been widely studied[4] and several fluoro-substituted compounds are now considered as analogs of plant hormones[5].

Several efforts for introducing fluorine groups in bioactive brassinosteroids have been made[6], but only a few studies on their properties have been reported[7], found that 25-fluor analogues of brassinolide and castasterone showed almost no activity while the presence of a 25-hydroxy group yields a molecule with potent biological activity.

Opposite results are found when the 5α-OH of 5α-hydroxy derivatives is substituted by 5α-fluor. Thus, whereas the 5α--fluor-28-homoteasterone does not elicit activity, similar activity is found for 5α-fluor-28-homocastasterone but an increase in the activity is observed in the case of 5α-fluor-28-homotyphasterol compared to the related 5α-OH analogs[9,11].

These unexpected results obtained while testing fluorina-

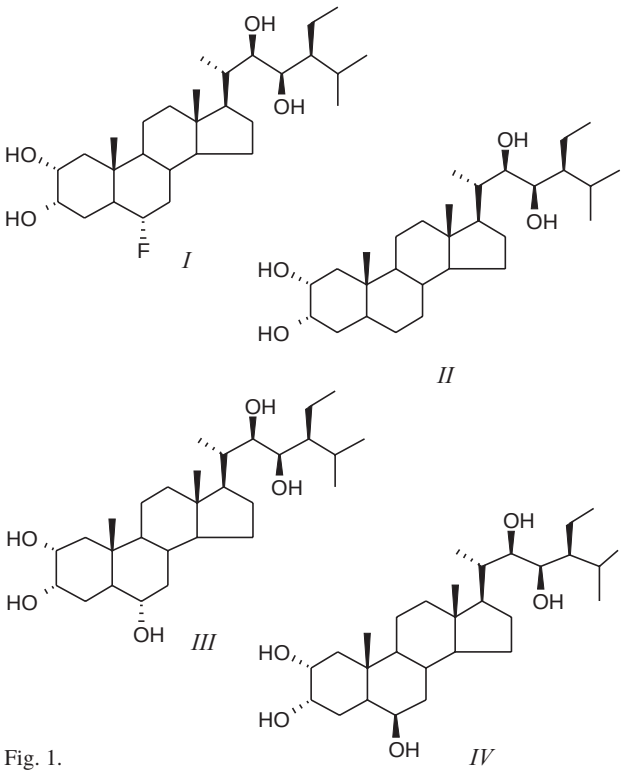


Fig. 1.

ted or hydroxylated brassinosteroids at different parts of the molecule reinforce the idea of the different mode of action of each functionality present in a brassinosteroid to elicit activity. To asses this, more analogues bearing both types of substituents should be analyzed.

In this communication we present the synthesis of a new C-6α fluorinated analogue (I), as well as the synthesis of other related analogues with or without hydroxyl groups at C-6: II, III and IV (Fig. 1). We also discuss the bioactivity exhibited by compounds I–IV in the Rice lamina inclination test.

REFERENCES

1. Liebman J., Greenberg A., Dolbier Jr. W.: *Fluorine-Containing Molecules: Structure, Reactivity, Synthesis* (Eswarakrishnan S., ed.). VCH, New York 1988.
2. Welch J., Eswarakrishnan S.: *Fluorine in Bioorganic Chemistry*. Wiley, New York 1991.
3. Filler R., Kobayashi Y., Yagupolskii L.: *Organofluorine Compounds in Medicinal Chemistry and Biomedical Applications*. Elsevier, Amsterdam 1993.
4. Kobayashi Y., Taguchi T.: Yakugaku Zasshi *120*, 951 (2000).
5. Saito T., Kamiya Y., Yamane H., Murofushi N., Sakurai A., Takahashi N.: Plant Cell Physiol. *39*, 574 (1998).
6. Jin F., Xu Y., Huang W.: J. Chem. Soc., Perkin Trans. 1 *1993*, 795.
7. Back T., Janzen L., Nakajima S., Pharis R.: J. Org. Chem. *64*, 5494 (1999).
8. Jiang B., Ying L., Zhou W.-S.: J. Org. Chem. *65*, 2631 (2000).
9. Ramírez A., Gros E., Galagovsky L.: Tetrahedron *56*, 6171 (2000).
10. Galagovsky L., Gros E., Ramírez A.: Phytochemistry *58*, 973 (2001).
11. Brosa C., Soca L., Terricabras E., Ferrer J. C., Alsina A.: Tetrahedron *54*, 12337 (1998).

ETHYLENE DOES NOT MEDIATE BRASSINOSTEROID-DRIVEN SHOOT PROLIFERATION IN *in vitro*-GROWN MARUBAKAIDO APPLE ROOTSTOCK

ADAUCTO B. PEREIRA-NETTO[a], SILVIA SCHAEFER[a], SANDRA A. MEDEIRO[a], CLAUDIA T. A. C. SILVA[a], LYDIA R. GALAGOVSKY[b], JAVIER A. RAMIREZ[b]

[a]Department of Botany-SCB, Politechnic Center, University of Paraná, C.P. 19031 81531-970 Curitiba-PR, Brazil, [b]Department of Organic Chemistry, School of Exact and Natural Sciences, University of Buenos Aires, Ciudad Universitaria, Pabellón 2, Buenos Aires, Argentina
E-mail: apereira@ufpr.br

Marubakaido *(Malus prunifolia*, Willd, Borkh), also known as maruba, chinese apple or plumleaf crabapple is the most widely used apple rootstock in countries such as Brazil. However, this rootstock is susceptible to various viruses, which make *in vitro* propagation of virus-free propagules one of the most recommended techniques for its multiplication, though the low *in vitro* multiplication rate typically found for the marubakaido make this propagation technique barely feasible for commercial purposes. We have previously shown that 5F-Homoethylcastasterone (5F-HCTS) (ref.[1]) is able to more than double the *in vitro* multiplication rate for marubakaido, mainly through a stimulation of lateral branch proliferation[2]. Since ethylene is thought to mediate at least some of the brassinosteroid-induced plant responses, we investigated a possible involvement of ethylene in the 5F-HCTS-driven lateral branch proliferation in the marubakaido apple rootstock. Application of 5F-HCTS at 500 $\mu g.shoot^{-1}$ resulted in increased ethylene release during the first eight days of culture. It was thus surprising to find that shoots grown in the presence of 1-aminocyclopropane-1-carboxylic acid, the immediate precursor of ethylene biosynthesis, (3–400 μM range), in the culture medium did not present branching stimulation. Moreover, shoots grown in ethylene-enriched atmosphere showed progressive branching inhibition with increasing ethylene concentrations (0.1–60 $\mu mol.l^{-1}$). In addition to that, branching stimulation was found for shoots grown in the presence of 1.25 μM aminoethoxyvinylglycine, an effective inhibitor of ethylene biosynthesis. When grown in atmosphere enriched with 1-methylcyclopropene (100 and 200 $\mu l.l^{-1}$), an inhibitor of the ethylene signal transduction pathway, marubakaido shoots presented a smaller number of branches measuring 15 mm in length or more, and a larger number of branches measuring less than 15 mm in length, compared to shoots grown in 1-methylcyclopropane-free atmosphere. These results indicate that ethylene probably is not a mediator of the brassinosteroid-driven branch proliferation in the marubakaido apple rootstock.

REFERENCES

1. Ramírez J. A., Gros E. G., Galagovsky L. R.: Tetrahedron *56*, 6171 (2000).
2. Schaefer S.; Medeiro S. A., Ramírez J. A., Galagovsky L. R., Pereira-Netto A. B.: Plant Cell Rep. *20*, 1093 (2002).

PUZZLING DATA FROM BRASSINOSTEROID BIOASSAYS

L. GALAGOVSKY[a], J. RAMÍREZ[a], S. CLOUSE[b], M. STRNAD[c], L. KOHOUT[d]

[a]Departamento de Química Orgánica; Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 1428, Buenos Aires, Argentina, [b]Department of Horticultural Science, North Carolina State University, Raleigh, North Carolina, 27695, USA, [c]Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Slechtitelu 11, Olomouc, [d]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: lyrgala@qo.fcen.uba.ar, steve_clouse@ncsu.edu, strnad@ueb.cas.cz, kohout@uochb.cas.cz

As brassinosteroids (BRs) are present in plants in extremely low concentrations, many of them have been prepared

synthetically to elucidate structure-activity relationships. Sensitive and specific bioassays are required in order to differentiate changes in bioactivity due to small changes in molecular structure. Many bioassays used for the other phytohormones have been described, but only few of them are in use for evaluation of brassinosteroids.

Khripach[1] reviewed papers on the bioactivity of BRs in different bioassays. Reports show that strict fulfillment of experimental conditions is necessary to obtain reproducible results. Differences in the cultivar of the plants, the conditions of germination and growth, the phase of development, the light conditions, and the mode of treatment were found to be of the utmost importance for each test.

A set of systems to assay BR activity in a variety of physiological responses including cell elongation, cell division and cell differentiation on *Arabidopsis thaliana* wild type and mutants has also been described[2].

The use of mutants of *A. thaliana* that are deficient in or sensitive to plant hormones has been invaluable in dissecting the molecular mechanisms of other phytohormones. Hormone-deficient mutants usually result from lesions in genes encoding hormone biosynthetic enzymes and are rescued to wild-type phenotype by treatment with the hormone. Studies of the growth responses to BR application in dwarfed *Arabidopsis thaliana det2* mutants revealed a prominent hypocotyl elongation, which can be used as a novel high specific and sensitive biotest for BRs.

This communication analyses data of nine structurally-related BRs in four different bioassays: Bean Second-Internode Bioassay (BSIB), Rice Lamina Inclination Test (RLIT), and Effects on Hypocotyls Growth (EHG) on both *A. thaliana* wild type and *det2* mutant.

Results confirm that minor changes in BR structure may lead to completely different results in different bioassays.

This work was supported by UBACyT X130 (Universidad de Buenos Aires, Argentina), and by projects Nos. 203/01/0083 and Z 4055905 (GA CR and IOCB).

REFERENCES

1. Khripach V. A., Zhabinskii V. N., de Groot A. E.: *Physiological Mode of Action of Brassinosteroids*, in *Brassinosteroids: A New Class of Plant Hormones* (Khripach V. A., Zhabinskii V. N., de Groot A. E., ed.), pp. 219–300. Academic Press, San Diego 1999.
2. Clouse S. D., Feldman K. A. *Molecular Genetics of Brassinosteroid Action*, in *Brassinosteroids: Steroidal Plant Hormones* (Sakurai A., Yokota T., Clouse S. D., ed.). Springer Verlag, Tokyo 1999.

ANTIVIRAL ACTIVITY OF BRASSINOSTEROIDS: *in vitro* AND *in vivo* STUDIES

LYDIA R. GALAGOVSKY[b], FLAVIA MICHELINI[a], JAVIER RAMIREZ[b], ALEJANDRO BERRA[c], ANDREA BRUTTOMESSO[b], MONICA B. WACHSMAN[a], LAURA E. ALCHE[a]

[a]Laboratorio de Virología, Departamento de Química Biológica, [b]Departamento de Química Orgánica. Facultad de Ciencias Exactas y Naturales, [c]Laboratorio de Inmunología Ocular, Facultad de Medicina, Universidad de Buenos Aires, Argentina
E-mail: wachsman@qb.fcen.uba.ar, lyrgala@qb.fcen.uba.ar

Brassinosteroids are a novel group of steroids that appear to be ubiquitous in plants and are essential for normal plant growth and development. The natural brassinosteroids and their synthetic analogues, that have been identified so far, have in common a 5α-cholestan skeleton and their structural variations come from the type, position and stereochemistry of functional groups present in the A and B rings and in the side chain[1,2].

In previous reports, we studied the antiviral activity of thirty-seven synthetic analogues of the natural brassinosteroid (24*S*)-ethyl-brassinone against HSV-1 and of eleven derivatives against arenaviruses. Several of the tested compounds showed selectivity indexes (SI) between 10 and 18 fold higher than ribavirin for Junin virus (JV) and a moderate activity against HSV-1 (ref.[3,4]). Twenty-seven derivatives were also assayed against measles virus and five compounds showed a good antiviral activity with SI values higher than ribavirin used as reference drug[5].

In this study we screened the *in vitro* antiviral activity of a group of derivatives against Poliovirus and Stomatitis Vesicular Virus (VSV). For that purpose Vero cell cultures were infected with each virus at a multiplicity of infection of 1, and after 1 hour adsorption at 37 ˚C the derivatives were added at a concentration of 40 μM and after 18 h post-infection (p.i.), supernatants were harvested and titrated by a plaque assay. Most of the assayed brassinosteroids are active against Poliovirus, four of them present a percentage of inhibition for VSV higher than 90 %.

It is interesting to notice that the most active antiviral derivatives bear a 22*S*, 23*S* dihydroxy configuration in the side chain, while 22*R*, 23*R* configuration is the best to obtain plant growth bioactivity.

In this study we are also presenting our results when testing the antiviral activity ot three brassinosteroids derivatives *in vivo*. We assayed compounds (22*S*,23*S*)-3β-bromo-5α,22,23--trihydroxystigmastan-6-one (6b), (22*S*,23*S*)-3β,5α,22,23-tetrahydroxy-stigmastan-6-one (7b) and (22*S*,23*S*)-5α-fluoro--3β-22,23-trihydroxystigmastan-6-one (12b), the most active derivatives against herpes simplex type 1 virus (HSV-1) *in vitro*[4], using the experimental model of murine herpetic stromal keratitis (HSK). HSK is an important cause of corneal blindness induced by HSV-1. The cause of the disease depends on both viral multiplication and immunological response of the host[6]. Seventy adult Balb/c mice were inoculated with 10^5 UFP/5 μl of HSV-1 KOS strain in their corneas. Six groups of

10 mice each received 20 μM of 6b, 7b and 12b three times a day for three consecutive days, beginning at 1 and 6 days p.i. Remaining animals were left as a control of HSV-1 infection. All mice were monitored during 10 days. At 10 days p.i., treatment with 6b, 7b and 12b administered at 1 day p.i. delayed and reduced the severity of HSK with respect to untreated infected mice, consisting mainly in inflammation, vascularization and necrosis. However, when eyes were washed with culture medium at 1, 2 and 3 days p.i., no differences in viral titers among samples from treated and untreated mice were observed. In the case of treatment at 6 days p.i, only 7b was able to diminish the incidence of disease at 8 days p.i., indicating an eventual anti-inflammatory activity of this derivative in the prevention of ocular disease.

REFERENCES

1. Ramírez J., Gros E., Galagovsky L.: Steroids *65*, 329 (2000).
2. Ramírez J., Gros E., Galagovsky L.: Tetrahedron *56*, 6171 (2000).
3. Wachsman M. B., López E. M. F., Ramírez J. A., Galagovsky L. R., Coto C. E.: Antivir. Chem. Chemother. *11*, 71 (2000).
4. Talarico L. B., Ramirez J. A., Galagovsky L. R., Wachsman M. B.: Medical Chem. Res. (2003), in press.
5. Wachsman M. B., Ramírez J. A., Galagovsky L. R., Coto C. E.: Antiviral Chem. Chemother. *13*, 61 (2002).
6. Pifarré M. P., Berra A., Coto C., Alché L.: Exp. Eye Res. *75*, 327 (2002).

NEW APPLICATIONS OF CIRCULAR DICHROISM SPECTROSCOPY

JACEK GAWRONSKI

Department of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

Circular dichroism (CD) spectroscopy is rooted in the structural studies of optically active natural products, including isoprenoids. Pioneering studies in this field by Moffitt, Woodward, Moscowitz, Klyne and Djerassi resulted in the formulation of the *octant rule*[1], the first empirical sector rule of CD spectroscopy. While the sector rules proved useful at the pioneering times (i.e. in the sixtieths and seventieths of the 20th century), their significance has faded away with the development of computational methods of theoretical chemistry. Nowadays, the approximate solution of the Rosenfeld equation for a given structure allows non-empirical correlation of the CD spectrum with the absolute configuration and/or conformation of the molecule, regardless its chromophoric nature. Both semiempirical (AM1 or PM3)[2] and DFT[3] methods are used, their usefulness depending on the molecule size and complexity of the structural problem to be solved.

There are may examples of this procedure in the literature concerning either the electronic CD (ECD)[4] or the vibrational CD (VCD)[5]. In the latter case the calculation of the VCD spectrum is implemented in the operational software of the commercial instrument.

Although the process of assigning molecular chirality (e.g. absolute configuration) by circular dichroism invariably involves both the experiment and the confrontation of the result with the theoretical model, in many cases the theoretical model for ECD can be simplified to the non-empirical *coupled oscillator model*[6], which makes the foundation of the *exciton-coupled CD* (ECCD) method of Harada and Nakanishi[7]. In this method the absolute configuration (or conformation) can be conveniently determined without resorting to time-consuming computations, by examining the geometry of the two (or more) interacting electric dipole transition moments[8]. The applications of the ECCD method are virtually countless and range from configurational studies of single molecules to the determination of the conformation of biopolymers.

An extension of the ECCD method is the *substituent-polarization model* (*allylic axial chirality*)[9] where one of the interacting electric dipole transition moments is replaced by the induced dipole of the (non-chromophoric) C-X bond (X = carbon, oxygen, nitrogen, halogen). Thus, the CD spectra of enones, dienes, butenolides and 3-pyrrolin-2-ones substituted in the allylic position have been analyzed by this method in order to establish the absolute configuration of the allylic carbon atom[10]. The usefulness of the CD method for the determination of absolute configuration is even more evident when the method of X-ray diffraction analysis cannot be applied, for example due to the unavailability of suitable monocrystal of the compound studied[11].

Stereostructures of monochromophoric or non-chromophoric molecules can also be analyzed by the ECCD method; the necessary chromophore(s) are introduced by derivatization of the non-chromophoric group(s). Groups such as hydroxy, amino and carboxy are suitable for chromophoric derivatization. To avoid interactions with other chromophores present in the investigated molecule, the reporting chromophores, when necessary, should have red-shifted principal absorption band[12].

In addition, non-chromophoric molecules having just one hydroxy or amino group can be analyzed by the ECCD method when derivatized with a chiral but racemic bichromophore. Steric effects in the derivatized molecule lead to desymmetrization of the equilibrium conformation of the bichromophore and to induced CD, which can be analyzed by the exciton coupling mechanism[12].

There has been long-standing dilemma of determining simultaneously the absolute configuration and conformation of a molecule from the CD spectrum. Whereas such an information is in principle contained in the CD spectrum, approximate methods of theoretical or geometrical analysis of the CD data do not allow generally to determine the absolute configuration without a priori knowledge of the relative conformation (this can be acquired for example, from the NMR spectra). Nevertheless, in some cases where the Cotton effects of a molecule can be analyzed according to two different mechanisms, both the absolute configuration and conformation can be determined from the CD data[13].

REFERENCES

1. (a) Moffitt W., Woodward W. B., Moscowitz A., Klyne

W., Djerassi C.: J. Am. Chem. Soc. *83*, 4013 (1961); (b) Djerassi C., in: *Optical Rotatory Dispersion and Its Applications to Organic Chemistry*, Chap. 13. McGraw Hill, New York 1960; (c) Lightner D. A., Gurst J. E., in: *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*, Chap. 4–10. Wiley-VCh, New York 2000; (d) Kirk D. N.: Tetrahedron *42*, 777 (1986).
2. For example: CAChe WS Pro 5.0, Fujitsu Ltd, 2001.
3. For example: Gaussian 98, Revision A10, Gaussian, Inc., Pittsburgh, PA 2001.
4. (a) Linker T., Rebien F., Tóth G., Simon A., Kraus J., Bringmann G.: Chem. Eur. J. *4*, 1944 (1998); (b) Bringmann G., Busemann S., Krohn K., Beckmann K.: Tetrahedron *53*, 1655 (1997).
5. (a) Devlin F. J., Stephens P. J., Cheeseman J. R., Frisch M. J.: J. Phys. Chem. A *101*, 9912 (1997); (b) Krebs A. W., Thólke B., Pforr K.-I., König W. A.; Scharwächter K., Grimme S., Vögtle F., Sobanski A., Schramm J., Hormes J.: Tetrahedron: Asymmetry *10*, 3483 (1999).
6. (a) Kuhn W.: Trans. Faraday Soc. *26*, 293 (1930); Annu. Rev. Phys. Chem. *9*, 417 (1958); (b) Mason S. F.: Proc. Chem. Soc. *1962*, 362.
7. (a) Harada N., Nakanishi K., in: *Circular Dichroic Spectroscopy:Exciton Coupling in Organic Stereochemistry*. University Science Books, Mill Valley 1983; (b) Nakanishi K., Berova N., in: *Circular Dichroism: Principles and Applications* (Nakanishi K., Berova N., Woody R. E., ed.), Chap. 13. VCH, Deerfield Beach 1994; (c) Berova N., Nakanishi K., in: *Circular Dichroism*: *Principles and Applications* (Berova N., Nakanishi K., Woody R. B., ed.), Chap. 12. Wiley-VCH, New York 2000.
8. Rodger A., Nordén B., in: *Circular Dichroism and Linear Dichroism*, Chap. 5 and 7. Oxford University Press, Oxford 1997.
9. (a) Kirkwood J. G.: J. Chem. Phys. *5*, 479 (1937); (b) Mason S. F., in: *Optical Activity and Chiral Discrimination* (Mason S. F., ed.), Chap. 1. Reidel D., Dordrecht 1979.
10. (a) Gawroński J. K.: Tetrahedron *38*, 3 (1982); (b) Gawroński J., in: *The Chemistry of Enones* (Patai S., Rapoport Z. ed.), pp. 55–105. Wiley, New York 1989; (c) Gawroński J., Gawrońska K., Buczak G., Katrusiak A., Skowronek P., Suemune H.: Tetrahedron: Asymmetry *7*, 301 (1996); (d) Gawroński J. K., in: *Circular Dichroism – Principles and Applications* (Berova N., Nakanishi K., Woody R.W., ed.), pp. 305–335. Wiley-VCh, New York 2000; (e) Gawroński J. K., van Oeveren A., van der Deen H., Leung C. W., Feringa B. L.: J. Org. Chem. *61*, 1513 (1996); (f) Cuiper D., Brzostowska M., Gawroński J. K., Smeets W. J. J., Spek A. L., Hiemstra H., Kellogg R. M., Feringa B. L.: J. Org. Chem. *64*, 2567 (1999).
11. Example – acetogenins: Gawroński J., Wu Y.-C.: Polish J. Chem. *73*, 241 (1999).
12. Gawroński J., Skowronek P.: Curr. Org. Chem. (2003), in press.
13. Gawroński J., Grycz P., Kwit M., Rychlewska U.: Chem. Eur. J. *8*, 4210 (2002).

SYNTHETIC APPROACHES TO THE MARINE DITERPENOIDS SARCODICTYINS, POTENT MICROTUBULE-STABILIZING ANTICANCER AGENTS

CESARE GENNARI

Dipartimento di Chimica Organica e Industriale, Centro di Eccellenza C.I.S.I., Universita' di Milano, Istituto CNR di Scienze e Tecnologie Molecolari, via Venezian 21, I-20133 Milano, Italy
E-mail: cesare.gennari@unimi.it

Sarcodictyins A and B 1 and eleutherobin 2 (the "eleutheside" family of microtubule-stabilizing agents, Fig. 1) are active against paclitaxel-resistant tumor cell lines and therefore hold potential as second generation microtubule-stabilizing anticancer drugs[1]. The scarce availability of 1–2 from natural sources makes their total syntheses vital for further biological investigations[1]. (For a comprehensive review on the chemistry and biology of the sarcodictyins, see[1]). To date, sarcodictyins A and B have been synthesized successfully by Nicolaou et al.[2], who have also exploited a similar route for accessing eleutherobin[3]. A subsequent report by Danishefsky and coworkers details an elegant alternative access to eleutherobin[4]. A number of partial syntheses and approaches have also been described[5].

The total syntheses of the eleuthesides have generated very limited diversity in the diterpenoid core, with major variations reported only in the C-15 functionality and C-8 side-chain[1–4]. In a recent communication[5h], we described the transformation of aldehyde 3 (prepared in 6 steps on a multigram scale from *R*-(–)-carvone in 30 % overall yield)[5a,g] into the RCM precursor 8 *via* multiple stereoselective Brown allylations[6] (Scheme 1). Diene 8 was subjected to ring closing metathesis[7] using the "second generation" RCM-catalysts[8] 10 and 11 to give the desired ring closed product 9 as a single *Z* stereoisomer in ≥80 % yield[5h].

As part of our ongoing program aimed at the synthesis of simplified analogues of the eleutheside natural products, ideally showing improved synthetic accessibility and retaining microtubule-stabilizing properties, I describe in this lecture

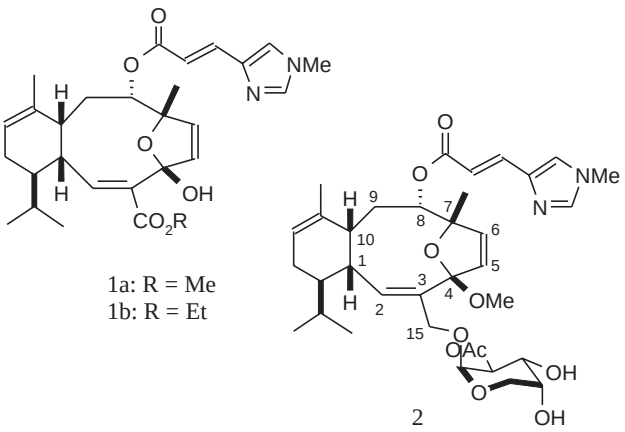
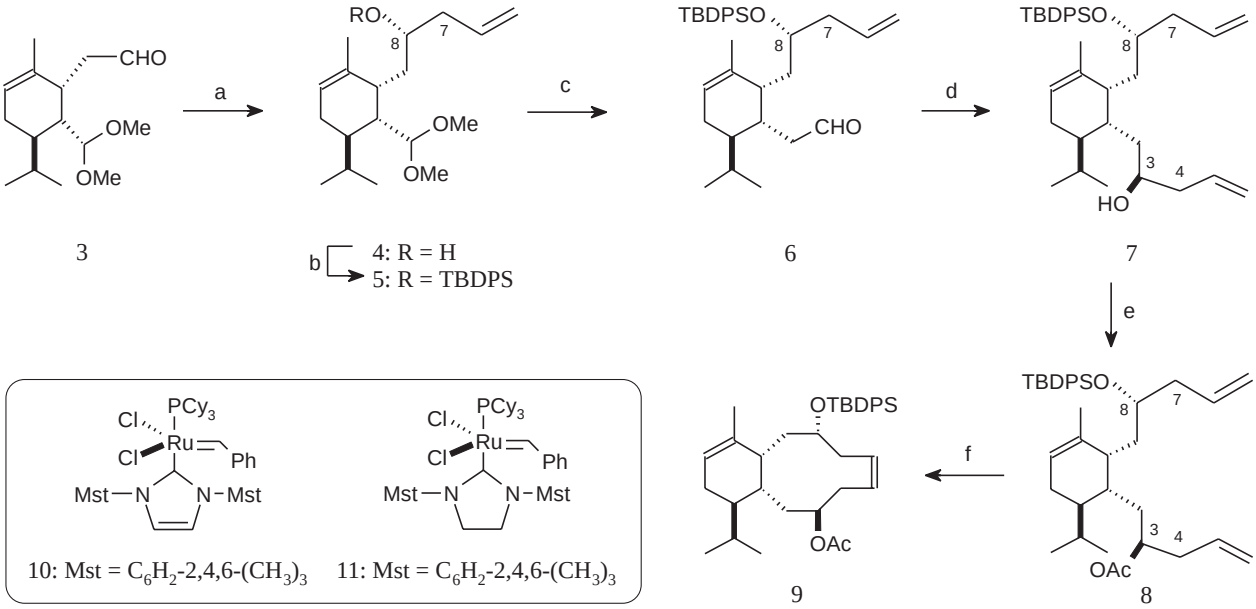
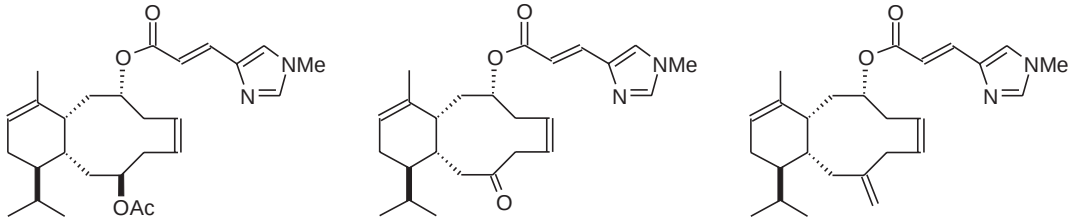


Fig. 1. Marine diterpenoids sarcodictyin A (1a), B (1b) and eleutherobin (2)



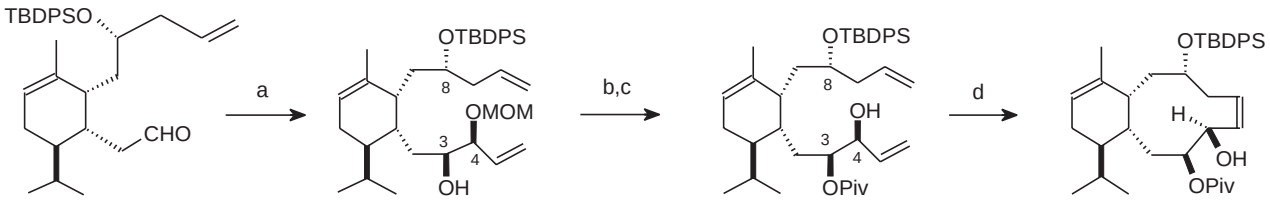
3 4 5 6 7 8 9

Scheme 1. Reagents and conditions: (a) i. AllMgBr, (l)-Ipc$_2$BOMe, Et$_2$O-THF, 0 ˚C to RT; ii. 3, –78 ˚C to RT, 6 h; iii. 6 N NaOH, H$_2$O$_2$, RT, 15 h, 77 % (>95% diastereomeric purity). (b) TBDPS-Cl, excess imidazole, CH$_2$Cl$_2$, RT, 16 h, 99%. (c) i. AcOH:THF:H$_2$O (3:1:1), RT, 16 h, 99%; ii. NaBH$_4$, EtOH, RT, 15 min, 98%; iii. MsCl, Et$_3$N, CH$_2$Cl$_2$, 0 ˚C to RT, 1 h, 99%; iv. KCN, 18-crown-6, MeCN, 80 ˚C, 5 h, 95%; v. DIBAl-H, hexane-toluene (2:1), –78 ˚C, 40 min, 99%. (d) i. AllMgBr, (l)-Ipc$_2$BOMe, Et$_2$O-THF, 0 ˚C to RT; ii. 6, –78 ˚C to RT, 2 h; iii. 6 N NaOH, H$_2$O$_2$, RT, 15 h, 55% (>95% diastereomeric purity). (e) Ac$_2$O, cat. DMAP, Py, RT, 94%. (f) 10 (30% mol), CH$_2$Cl$_2$, RT, 24 h, 80% (100% Z), or 11 (7% mol), CH$_2$Cl$_2$, RT, 168 h, 88% (95% after recovering starting material, 100% Z)



12 13 14

Fig. 2. Eleutheside analogues 12–14



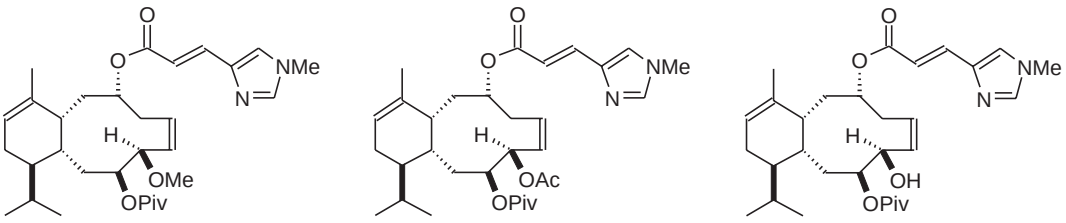
6 15 16 17

Scheme 2. Reagents and conditions: (a) i. (l)-Ipc$_2$BOMe, AllO-MOM, *s*-BuLi, BF$_3$-Et$_2$O, THF, –78 ˚C; ii. 6 N NaOH, H$_2$O$_2$, RT, 15 h, 77% (91% diastereomeric purity). (b) *t*-BuCOCl (PivCl), cat. DMAP, Py, RT, 80%. (c) BF$_3$-Et$_2$O, PhSH, CH$_2$Cl$_2$, –78 to –10 ˚C, 64%. (d) 11 (10% mol), CH$_2$Cl$_2$, RT, 120 h, 73% (100% Z)

the synthesis of a number of eleutheside analogues with potent tubulin-assembling and microtubule-stabilizing activity, using ring closing metathesis as the key-step for obtaining the 6–10 fused bicyclic ring system[5j]. A first set of eleutheside analogues (12–14) was synthesized from compound 9 using standard, high-yielding transformations (Fig. 2).

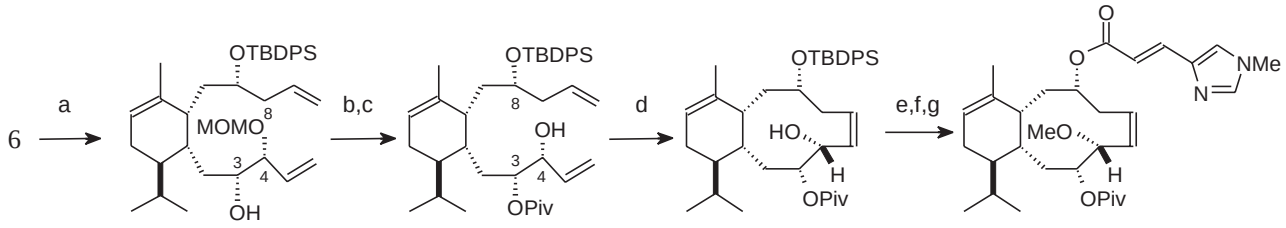
Aldehyde 6 was oxyallylated using Brown's methodology [(*Z*)-γ-(Methoxymethoxy) allyldiisopinocampheyl-borane from (–)-α-pinene][6d] in high yield (77 %) and with good stereoselectivity (3*S*,4*S*:3*R*,4*R* = 91:9, Scheme 2).

The major diastereomer 15 was isolated by flash chromatography and transformed into the allylic alcohol 16 *via* a sim-



18 19 20

Fig. 3. Eleutheside analogues 18–20



21 22 23 24

Scheme 3. Reagents and conditions: (a) i. (d)-Ipc$_2$BOMe, AllO-MOM, *s*-BuLi, BF_3-Et_2O, THF, –78 °C; ii. H_2O_2, 6 N NaOH, RT, 15 h, 76% (97.4% diastereomeric purity). (b) *t*-BuCOCl (PivCl), cat. DMAP, Py, RT, 94%. (c) BF_3-Et_2O, Me_2S, CH_2Cl_2, –20 °C, 78%. (d) 11 (10% mol), CH_2Cl_2, RT, 120 h, 60% (100% Z). (e) MeOTf, 2,6-di-*t*-Bu-Py, CH_2Cl_2, 40 °C, 99%. (f) TBAF, THF, RT, 67%. (g) mixed anhydride (ref. 2b), Et_3N, DMAP, $ClCH_2CH_2Cl$, 79%

ple protection/deprotection sequence. "Second generation" Grubbs' catalyst 11 gave the desired ring closed product 17 as a single *Z* stereoisomer in 73 % yield. As expected, entropic support (by virtue of the *cis* fusion to the cyclohexyl ring) made ring closure of diene 16 extremely smooth. Luckily, and delightfully, the stereochemistry of the double bond created by the RCM reaction was fully controlled in the desired sense (100 % *Z*) by the structure of the new 10-membered ring[9]. (Application of the RCM reaction to 10-membered carbocycles is still very rare, see[9]). The stereochemical course likely reflects thermodynamic control[10]. (The use of "second generation" metathesis catalysts results in the selective formation of the thermodynamically favored stereoisomeric products in RCM reactions furnishing medium-sized rings, see[10].) The crucial role of the protecting groups in the cyclization precursor 16 is noteworthy: (*a*) the large TBDPS group in position 8 helps to suppress the undesired dimerization reaction[11]; (*b*) a free allylic alcohol[12] in position 4 is necessary to promote the cyclization (the RCM reaction did not occur on dienes with variously protected allylic alcohols in position 4)[13]. (For discussions on the role of allylic oxygen substituents in the RCM reaction, see[13].) Compound 17 was transformed into a second set of eleutheside analogues 18–20, using standard, high-yielding transformations (Fig. 3).

Aldehyde 6 was also oxyallylated using Brown's enantiomeric reagent [(*Z*)-γ-(Methoxymethoxy)allyldiiso-pinocampheyl-borane from (+)-α-pinene][6d] in high yield (76 %) and with excellent stereoselectivity (3*R*,4*R*:3*S*,4*S* = 97.4:2.6, Scheme 3).

The major diastereomer 21 was isolated by flash chromatography and transformed into the allylic alcohol 22 *via* a simple protection/deprotection sequence (in this case Me_2S, $BF_3.Et_2O$ proved more reliable than PhSH, $BF_3.Et_2O$ for deprotecting the allylic alcohol from the MOM group)[14]. Treatment with catalyst 11 gave the desired ring closed product 23 as a single *Z* stereoisomer in 60 % yield. Finally, a standard sequence of reactions transformed compound 23 into the eleutheside analogue 24. The effect of these new eleutheside analogues on the assembly of tubulin was assessed at Pharmacia (Nerviano, Italy) and at Salford (UK), using paclitaxel as a reference (Table I).

Eleutheside analogue 13 was shown to be at least as potent as paclitaxel. Microtubules were generated in the presence of

Table I
Tubulin polymerizing activities (ED50 = effective dose that induces 50 % tubulin polymerization; ED90 = effective dose that induces 90 % tubulin polymerization (see: Battistini C., Ciomei M., Pietra F., D'Ambrosio M., Guerriero A. (Pharmacia): PCT Int. Appl. WO 96 36,335, 1996; Chem. Abstr. *126*, P54863x (1997)). ED values may vary depending on the tubulin batch (from pig brain): the same batch is used for the paclitaxel reference assay)

Compound	ED50 [μM]	ED90 [μM]	Paclitaxel ED50 [μM]	Paclitaxel ED90 [μM]
12	2.0	10.0	<0.5	0.5
13	0.2	1.2	0.5	3.0
14	5.0	16.0	<0.5	0.5
18	3.0	7.0	0.5	3.0
19	1.0	1.7	<0.5	0.5
20	1.0	1.8	<0.5	0.5
24	<0.5	1.0	<0.5	1.0

$CaCl_2$ and were stable (did not depolymerize) at 10 °C. Although there is a general agreement that the (*E*)-*N*-methylurocanic side chain, the C-4/C-7 ether bridge, and the cyclohexene ring are important determinants of antimitotic activity,[1] it is interesting to note that these simplified analogues of the natural product (lacking *inter alia* the C-4/C-7 ether bridge) retain potent microtubule stabilizing activity. Given the dramatic impact that the furanose oxygen deletion is likely to have on the conformation of the ring system, the fact that some of these compounds retain activity comparable to paclitaxel in the tubulin polymerization assay is remarkable. Work is in progress to synthesize more potent eleutheside analogues, investigate the interaction with tubulin and establish their cytotoxicity.

We thank the European Commission for financial support (IHP Network grant "Design and synthesis of microtubule stabilizing anticancer agents" HPRN-CT-2000-00018) and for postdoctoral fellowships to L. Caggiano, R. Beumer, S. Ducki (HPRN-CT-2000-00018), P. Bayón ("Marie Curie" HPMF--CT-2000-00838) and J. Telser ("Marie Curie" HPMF-CT--1999-00001). We also like to thank Merck (Merck's Academic Development Program Award to C. Gennari, 2001-02) and M.U.R.S.T. COFIN 2000 (MM03155477) for financial support.

REFERENCES

1. (a) Nicolaou K. C., Pfefferkorn J., Xu J., Winssinger N., Ohshima T., Kim S., Hosokawa S., Vourloumis D., van Delft F., Li T.: Chem. Pharm. Bull. *47*, 1199 (1999); (b) Britton R., de Silva D. E., Bigg C. M., McHardy L. M., Roberge M., Andersen R.: J. Am. Chem. Soc. *123*, 8632 (2001) and references therein.
2. (a) Nicolaou K. C., Xu J.-Y., Kim S., Ohshima T., Hosokawa S., Pfefferkorn J.: J. Am. Chem. Soc. *119*, 11353 (1997); (b) Nicolaou K. C., Xu J. Y., Kim S., Pfefferkorn J., Ohshima T., Vourloumis D., Hosokawa S.: J. Am. Chem. Soc. *120*, 8661 (1998); (c) Nicolaou K. C., Kim S., Pfefferkorn J., Xu J., Ohshima T., Hosokawa S., Vourloumis D., Li T.: Angew. Chem., Int. Ed. Engl. *37*, 1418 (1998).
3. (a) Nicolaou K. C., van Delft F., Ohshima T., Vourloumis D., Xu J., Hosokawa S., Pfefferkorn J., Kim S., Li T.: Angew. Chem., Int. Ed. Engl. *36*, 2520 (1997); (b) Nicolaou K. C., Ohshima T., Hosokawa S., van Delft F. L., Vourloumis D., Xu J. Y., Pfefferkorn J., Kim S.: J. Am. Chem. Soc. *120*, 8674 (1998).
4. (a) Chen X.-T., Gutteridge C. E., Bhattacharya S. K., Zhou B., Pettus T. R. R., Hascall T., Danishefsky S. J.: Angew. Chem., Int. Ed. Engl. *37*, 185 (1998); (b) Chen X.-T., Zhou B., Bhattacharya S. K., Gutteridge C. E., Pettus T. R. R., Danishefsky S. J.: Angew. Chem., Int. Ed. Engl. *37*, 789 (1998); (c) Chen X.-T., Bhattacharya S. K., Zhou B., Gutteridge C. E., Pettus T. R. R., Danishefsky S. J.: J. Am. Chem. Soc. *121*, 6563 (1999).
5. (a) Ceccarelli S., Piarulli U., Gennari C.: Tetrahedron Lett. *40*, 153 (1999); (b) Baron A., Caprio V., Mann J.: Tetrahedron Lett. *40*, 9321 (1999); (c) Carter R., Hodgetts K., McKenna J., Magnus P., Wren S.: Tetrahedron *56*, 4367 (2000); (d) Ceccarelli S., Piarulli U., Gennari C.: J. Org. Chem. *65*, 6254 (2000); (e) Xu Q., Weeresakare M., Rainier J. D.: Tetrahedron *57*, 8029 (2001); (f) Ceccarelli S., Piarulli U., Telser J., Gennari C.: Tetrahedron Lett. *42*, 7421 (2001); (g) Ceccarelli S., Piarulli U., Gennari C.: Tetrahedron *57*, 8531 (2001); (h) Telser J., Beumer R., Bell A. A., Ceccarelli S. M., Monti D., Gennari C.: Tetrahedron Lett. *42*, 9187 (2001); (i) Sandoval C., Redero E., Mateos-Timoneda M. A., Bermejo F. A.: Tetrahedron Lett. *43*, 6521 (2002); (j) Beumer R., Bayón P., Bugada P., Ducki S., Mongelli N., Sirtori F. R., Telser J., Gennari C.: Tetrahedron Lett. *44*, 681 (2003).
6. (a) Brown H. C., Desai M. C., Jadhav P. K.: J. Org. Chem. *47*, 5065 (1982); (b) Brown H. C., Jadhav P. K.: J. Am. Chem. Soc. *105*, 2092 (1983); (c) Jadhav P. K., Bhat K. S., Perumal P. T., Brown H. C.: J. Org. Chem. *51*, 432 (1986); (d) Brown H. C., Jadhav P. K., Bhat K. S.: J. Am. Chem. Soc. *110*, 1535 (1988); (e) Brown H. C., Racherla U. S., Liao Y., Khanna V. V.: J. Org. Chem. *57*, 6608 (1992).
7. (a) Grubbs R. H., Chang S.: Tetrahedron *54*, 4413 (1998); (b) Armstrong S. K.: J. Chem. Soc., Perkin Trans. 1 *1998*, 371; (c) Maier M. E.: Angew. Chem., Int. Ed. Engl. *39*, 2073 (2000); (d) Fürstner A.: Angew. Chem., Int. Ed. Engl. *39*, 3012 (2000).
8. (a) Huang J., Stevens E. D., Nolan S. P., Petersen J. L.: J. Am. Chem. Soc. *121*, 2674 (1999); (b) Scholl M., Trnka T. M., Morgan J. P., Grubbs R. H.: Tetrahedron Lett. *40*, 2247 (1999); (c) Huang J., Schanz H.-J., Stevens E. D., Nolan S. P.: Organometallics *18*, 5375 (1999); (d) Scholl M., Ding S., Lee C. W., Grubbs R. H.: Org. Lett. *1*, 953 (1999); (e) Trnka T. M., Grubbs R. H.: Acc. Chem. Res. *34*, 18 (2001).
9. (a) Nevalainen M., Koskinen A. M. P.: Angew. Chem., Int. Ed. Engl. *40*, 4060 (2001); J. Org. Chem. *67*, 1554 (2002); (b) ref. 5h, 5j.
10. (a) Fürstner A., Radkowski K., Wirtz C., Goddard R., Lehmann C. W., Mynott R.: J. Am. Chem. Soc. *124*, 7061 (2002); (b) Murga J., Falomir E., Garcìa-Fortanet J., Carda M., Marco J. A.: Org. Lett. *4*, 3447 (2002).
11. Buschmann N., Rückert A., Blechert S.: J. Org. Chem. *67*, 4325 (2002).
12. Hoye T. R., Zhao H.: Org. Lett. *1*, 1123 (1999).
13. (a) White J. D., Hrnciar P.: J. Org. Chem. *65*, 9129 (2000); (b) Paquette L. A., Efremov I.: J. Am. Chem. Soc. *123*, 4492 (2001); (c) Maishal T. K., Sinha-Mahapatra D. K., Paranjape K., Sarkar A.: Tetrahedron Lett. *43,* 2263 (2002).
14. (a) Fuji K., Kawabata T., Fujita E.: Chem. Pharm. Bull. *28*, 3662 (1980); (b) Sasaki M., Noguchi T., Tachibana K.: J. Org. Chem. *67*, 3301 (2002).

COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL FROM SOME CULINARY HERBS WILD GROWING IN CAMPANIA (SOUTHERN ITALY)

ARMANDO GRASSIA, CARMEN FORMISANO, DANIELA RIGANO, FELICE SENATORE

Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Via Domenico Montesano, 49-I 80131 Napoli, Italy
E-mail: fenciclidina@libero.it

Lamiaceae family is noteworthy for the number of species producing essential oils, spices or both. More of the plants that grow wild in Campania (Southern Italy) belongs to this family and are used in the popular medicine as depurative, diuretic, anti-varicose, in the treatment of colitis, bronchial asthma and catarrh and in the feeding[1,2]. The growing interest in the use of essential oils makes increasingly important the knowledge of their chemical composition and antibacterial activity. Our research group studies the plants of Southern Italy[3,4] and now we report the chemical composition and the antibacterial activity of *Ocimum basilicum* L., *Mentha piperita* L., *Salvia officinalis* L., *Thymus pulegioides* L., herbs widely used in Campania. Plants were collected in the "Parco Nazionale del Cilento" (Salerno, Southern Italy) and the essential oil from the fresh aerial parts of the plant was obtained by hydrodistillation and analysed by GC and CG/MS[3]. The antibacterial activity was evaluated by determining the MIC and the MBC using the broth dilution method[5]. Eight bacteria species, selected as representative of the class of Gram+ and Gram–, were tested: *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi Ty2*. Oil from *M. piperita* was characterized by high content of *trans*-menthone, *trans*-menthol and *trans*-menthyl acetate. Methyl chavicol was the main constituent of the essential oil from *O. basilicum*. α- and β-thujone were the major components of the *S. officinalis* essential oil. Oil from *T. pulegioides* was characterized by high content of thymol and its precursors *p*-cymene and γ-terpinene. Therefore the plant examined belongs to the chemotype rich in phenol compounds[6]. The values of MIC and MBC were generally at the same level and comparatively the oils were more active against Gram–positive bacteria, as evidenced by the lower MIC values. The results show that among the Gram+ bacteria, *S. faecalis* is the less sensitive to the effect of the oils. Among Gram– bacteria *P. aeruginosa* is very little or not affected by the oils (MIC values 100 or >100 $\mu g.ml^{-1}$). The time oil was the most active and *B. subtilis*, *S. aureus* and *E. coli* were the most susceptible to the biocidal effect of this oil. The sage oil was inactive (>100 $\mu g.ml^{-1}$) or very little active (100 $\mu g.ml^{-1}$). Also the basil oil is inactive or very little active on the tested bacteria except for *E. coli* (25 $\mu g.ml^{-1}$). The mint oil shows a very good activity (12.5 $\mu g.ml^{-1}$) against *B. subtilis* while the activity against the other micro-organisms is lower.

REFERENCES

1. Senatore F., De Feo V.: Fitoterapia *36*, 337 (1992).
2. De Feo V., Senatore F.: J. Etnopharm. *39*, 39 (1993).
3. Senatore F., Bruno M.: Flavour Frag. J. *18*, 195 (2003).
4. De Feo V., Bruno M., Senatore F.: J. Agric. Food Chem. (2003), in press.
5. Barry S., in: *The Antimicrobic Susceptibility Test: Principles and Practices*. Lea and Febiger, Philadelphia 1976.
6. Senatore F., in: *Oli essenziali – Provenienza, estrazione ed analisi chimica*. E.M.S.I., Roma 2000.

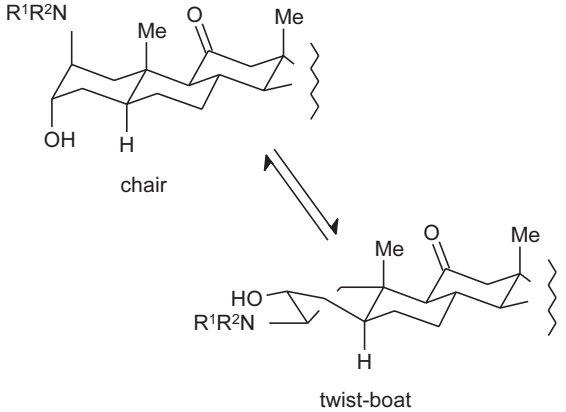
STEROIDS IN ANAESTHESIOLOGY: CHELATING AGENTS THAT REVERSE STEROID-INDUCED MUSCLE RELAXATION AND WATER SOLUBLE ANAESTHETIC STEROIDS

NIALL M. HAMILTON, ANDREW C. BOYD, ANTONIUS BOM, HELEN FIELDEN, DAVID K. GEMMELL, MAURICE S. MAIDMENT, ROSS McGUIRE

Departments of Medicinal Chemistry and Pharmacology, Organon Laboratories Ltd., Newhouse, Lanarkshire ML1 5SH, Scotland, UK
E-mail: n.hamilton@organon.co.uk

Steroid muscle relaxants like rocuronium bromide are employed by anaesthetists to facilitate tracheal intubation and prevent involuntary movements during surgery. Muscle relaxants act by blocking the effect of acetylcholine at nicotinic acetylcholine receptors. In general muscle relaxants have a short duration of action but sometimes more rapid reversal of this action is desirable. Unfortunately all current drugs to reverse paralysis are acetylcholinesterase inhibitors and produce serious side effects. Organon is developing Org 25969, a novel γ-cyclodextrin derivative that effects reversal of muscle relaxation by complexing with rocuronium bromide. The conformational changes necessary for the steroid to form a tight inclusion complex with Org 25969, which include ring A adopting a twist-boat form rather than a chair form (shown below), will be presented.

Several steroids have been evaluated in humans as potential intravenous anaesthetics but none is currently available due to side effects and/or prolonged duration/recovery. Some preliminary studies undertaken to evaluate ante (soft) steroids as potential new anaesthetics will be presented. In particular



it will be shown that steroids incorporating soft esters retain good *in vitro* activity at the $GABA_A$ receptor complex and good anaesthetic activity in mice. This profile is complemented by the relative inactivity of the metabolites that would arise from ester hydrolysis.

PHOTOCHEMICALLY-TRANSFORMED ECDYSTEROIDS AND THEIR INTERACTION WITH THE *Drosophila* ECDYSTEROID RECEPTOR

JURAJ HARMATHA[a], KAREL VOKÁČ[a], MILOŠ BUDĚŠÍNSKÝ[a], LAURENCE DINAN[b]

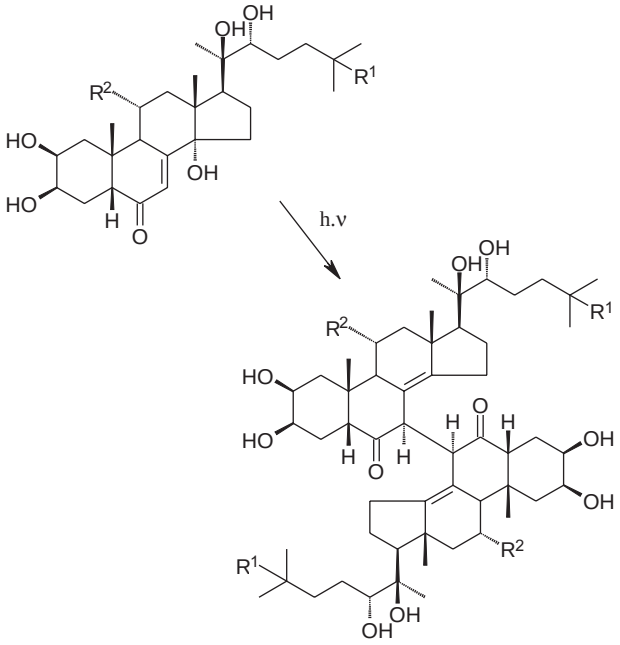
[a]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic, [b]Department of Biological Sciences, University of Exeter, Hatherly Laboratories, Prince of Wales Road, Exeter, EX4 4PS, U.K.
E-mail: harmatha@uochb.cas.cz

Ecdysteroids interact with the ligand-binding site of the ecdysteroid receptor mostly as agonists, but one analogue (a side-chain-modified lactone derivative) was found to demonstrate antagonistic activity. These activities can be detected and quantified with the *Drosophila melanogaster* B_{II} cell bioassay[1]. Biological activities of a series of natural and chemically modified ecdysteroids from our laboratory have been compared in this bioassay[2]. The natural ecdysteroids were isolated from higher plants, mainly from *Leuzea carthamoides*[3,4] or from fungi[5,6]. The structural analogues were prepared by chemical transformations[7] and the first dimeric ecdysteroid 4 was obtained by photochemical transformation[8] of 20-hydroxyecdysone (1, R^1 = OH, R^2 = H). The biological activity data were used to investigate structure-activity relationships[9], and for designing further targeted structural modifications[10]. The activities generally confirmed the activities in previous *in vivo* tests, with the exception of the dimeric ecdysteroids, which have not so far been assessed *in vivo*. In order to extend this study, we prepared and tested several new specific analogues and the ecdysteroid dimers 5 and 6 obtained by photochemical transformation of ponasterone A (2, R^1 = R^2 = H) and ajugasterone C (3, R^1 = H, R^2 = OH), respectively.

Differences in the solubility of the ecdysteroids 1–3 used for the transformation (i.e. various concentrations of reactants in the photoreactor) and the observed differences in yields of the dimers 4–6, led us to investigate the influence of concentration on the quantitative and qualitative content of photochemical transformation products. Experiments with oxygen in the reaction mixture (used instead of the inert argon protection) indicated a greater influence on the reaction product composition than did the reactant concentration.

We also compare the activities of the relatively large dimeric ecdysteroids with their structurally related, but considerably smaller, monomeric analogues. The results lead to the correction of some former general presumptions concerning ecdysteroid structure activity relationships.

Supported by GA CR, grant No. 203/01/018 and in part from research project Z4 055 905.



R^1	R^2	
OH	H	20-hydroxyecdysone (1)
OH	H	7,7'-bis-8(14)-ene-14-deoxy-20-hydroxyecdysone (4)
H	H	ponasterone A (2)
H	H	7,7'-bis-8(14)-ene-14-deoxy-ponasterone A (5)
H	OH	ajugasterone C (3)
H	OH	7,7'-bis-8(14)-ene-14-deoxy-ajugasterone C (6)

REFERENCES

1. Clément C. Y., Bradbrook D. A., Lafont R., Dinan L.: Insect Biochem. Mol. Biol. *23*, 187 (1993).
2. Harmatha J., Dinan L.: Arch. Insect Biochem. Physiol. *35*, 219 (1997).
3. Píš J., Budĕšínský M., Vokáč K., Laudová V., Harmatha J.: Phytochemistry *37*, 707 (1994).
4. Vokáč K., Budĕšínský M., Harmatha J.: Collect. Czech. Chem. Commun. *67*, 124 (2002).
5. Vokáč K., Budĕšínský M., Harmatha J., Píš J.: Tetrahedron *54*, 1657 (1998).
6. Vokáč K., Budĕšínský M., Harmatha J., Kohoutová J.: Phytochemistry *49*, 2109 (1998).
7. Píš J., Hykl J., Budĕšínský M., Harmatha J.: Tetrahedron *50*, 9679 (1994).
8. Harmatha J., Budĕšínský M., Vokáč, K.: Steroids *67*, 127 (2002).
9. Harmatha J., Dinan L., Lafont R.: Insect Biochem. Mol. Biol. *32*, 181 (2002).
10. Dinan L., Horman R. E., Fujimoto T.: J. Computer Aided Molec. Design *13*, 185 (1999).

ECDYBASE [http://ecdybase.org] – THE 2003 UPDATE OF THE NATURAL ECDYSTEROID DATABASE

RENÉ LAFONT[a], JURAJ HARMATHA[b], LAURENCE DINAN[c]

[a]Laboratoire d'Endocrinologie Moléculaire et Evolution, Université Pierre et Marie Curie, 75252 Paris 05, France, [b]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic, [c]Department of Biological Sciences, University of Exeter, Exeter, EX4 4PS, U.K
E-mail: rene.lafont@snv.jussieu.fr, harmatha@uochb.cas.cz, L.N.Dinan@exeter.ac.uk

The Ecdysone Handbook, originally created by René Lafont and Ian D. Wilson, was published as a hard copy in 1992 (1st Edition)[1] providing general data on 170 natural ecdysteroids. Each data file contained (whenever available) biological, chemical, structural, spectroscopic (UV, IR, MS, NMR) and chromatographic data together with selected relevant references. The ecdysteroid family, however, continued to increase in the next years and the enlarged 2nd Edition was published in 1996 (with 262 compounds)[2]. The number of compounds is, however, still growing (e.g., 312 compounds at the onset of 2000), and thus requiring new updated Editions. Owing to the limited number of ecdysteroid research specialists interested in such Handbooks, a printed version was no longer justified. Moreover, the problem of updating content was a major one, too. This led to the idea of transforming the Handbook in a Database made freely available on the web to anyone interested in ecdysteroids. The collected previous data with the new updated ones (Pagemaker® files) were converted to the "Ecdybase", presented in 2002 at the 15th Ecdysone Workshop[3] and opened to free access on the URL http://ecdybase.org. The design of the web interface, the server operation and the customer access statistics (impact) are operated by Cybersales a.s.

The present update and upgrade of the "Ecdybase" includes data on the biological activity of ecdysteroids (when available) and a catalogue of commercial products containing ecdysteroids, with direct links to the homepages of the relevant producers.

There are improved functions allowing e.g. a search of compounds by name or partial name (resulting in a list of all compounds with a name or partial name containing a string of at least 3 characters). The search is case-insensitive; thus, even an incorrect name transcription would result in a correct search. The present system allows searching by molecular weight, by molecular formula $C_{nx}H_{ny}O_{nz}$ (or selectively by the number of elements C_{nx}/O_{nz}), and by occurrence in biological sources (the search is again case-insensitive and set on a minimum 3 characters, allowing use of partial or incorrect names of species). Searching is also possible by the name of author (selected from an available list of quoted authors). Forms are now being prepared to enable researchers to submit electronically additional data for new ecdysteroids or to update information on already known compounds.

Partly supported by GA CR, grant No. 203/01/0183 and by the research project Z4 055 905.

REFERENCES

1. Lafont R., Wilson I. D.: *The Ecdysone Handbook*, 1st Edition, pp. 1–392. The Chromatographic Society Press, Nottingham 1992.
2. Lafont R., Wilson I. D.: *The Ecdysone Handbook*, 2nd Edition, pp. 1–525. The Chromatographic Society Press, Nottingham 1996.
3. Lafont R., Harmatha J., Marion-Poll F., Dinan L., Wilson I. D.: *Ecdybase – The Ecdysone Handbook*, 3rd Edition. Cybersales, Prague 2002; http://ecdybase.org, 9/6 2003.

SYNTHESIS OF FLUORINATED STEROIDS USING A NOVEL FLUORINATING REAGENT TAMPS

PAVEL HERRMANN[a], JAROSLAV KVÍČALA[b], HANA CHODOUNSKÁ[a*]

[a]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, [b]Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic
E-mail: hchod@uochb.cas.cz

In the course of previous research on neurosteroids was found that fluorine atom could effectively substitute a hydroxy function without harming the biological activity[1]. This leads to improved pharmacological characteristics of such derivatives, namely to enhanced metabolic stability. Synthesis of secondary fluoroderivatives by means of currently available nucleophilic fluorinating agents (e.g. DAST or TBAF) is not efficient enough as it is hampered by competing elimination.

Hence, the aim of our work was to assess the fluorinating ability of a recently developed nucleophillic fluorinating agent, tetrabutylammonium difluorodimethylphenyl- silicate (TAMPS), in fluorination of selected secondary tosylates and to compare the results with literature. These substrates were prepared following published procedures. The reagent itself has been developed and tested on aliphatic substrates by Kvíčala[2] and his coworkers (Fig. 1).

Fluorosteroids 1–10 (Fig. 2) were obtained from the corresponding tosylates after 4–6 h of reflux in acetonitrile under an argon atmosphere, using 2 equivalents of TAMPS (a solution in acetonitrile). Our results indicate that the fluorination into positions 3 and 7 proceeds stereoselectively with inversion of configuration and furnishes diastereoisomerically pure fluoroderivates in yields ranging from 20 % to 39 %, accom-

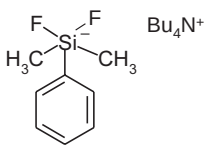
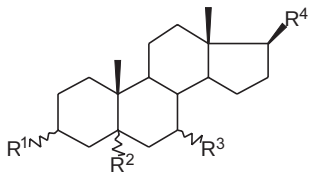
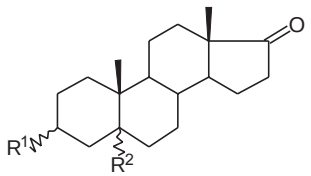


Fig. 1.



	R^1	R^2	R^3	R^4
1	α-F	α-H	H	C_8H_{17}
2	β-F	α-H	H	C_8H_{17}
3	α-F	β-H	H	C_8H_{17}
4	β-F	β-H	H	C_8H_{17}
5	β-OAc	α-H	α-F	C_8H_{17}
6	α-F	β-H	H	$COCH_3$



	R^1	R^2
7	α-F	α-H
8	β-F	α-H
9	α-F	β-H
10	β-F	β-H

Fig. 2.

panied by mixture of corresponding olefins as major products, whereas attempts to introduce fluorine into position 17 lead to mere elimination.

To sum up, the yields are comparable with DAST or TBAF, the stereoselectivity is superior to TBAF and the fluorination does not require any special equipment. Moreover, the TAMPS reagent is less expensive than DAST and safe to use.

The project was supported by research project Z4 055 905 and GA CR grant No. 203/01/0084 and 203/00/1232 of the GA CR.

REFERENCES

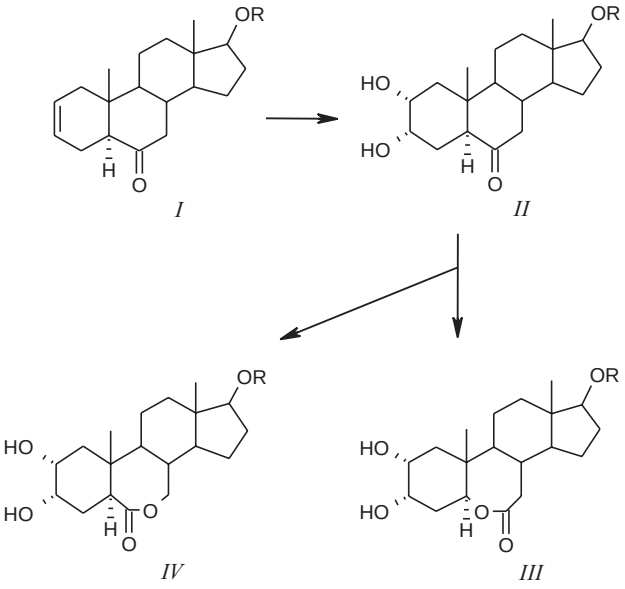
1. Slavíková B., Kasal A., Uhlířová L., Kršiak M., Chodounská H., Kohout L.: Steroids *66*, 99 (2001).
2. Kvíčala J., Mysík P., Paleta O.: Synlett *2001*, 547.

SYNTHESIS OF NEW BRASSINOSTEROIDS WITH VARIOUS ESTER GROUPS IN POSITION 17β

JAROSLAVA HNILIČKOVÁ, LADISLAV KOHOUT

Institute of Organic Chemistry and Biochemistry, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: jarka@uochb.cas.cz, kohout@uochb.cas.cz

In the course of our structure-activity relationships studies on brassinosteroids[1] we prepared series of brassinosteroids analogues with various ester functions in the position 17β. Starting material, 17β-hydroxy-5α-androst-2-en-6-one (*Ia*), was treated with appropriate acid anhydride to afford corresponding esters in the position 17β (*Ib–d*) which after hydroxylation with osmium tetroxide in the presence of *N*-methylmorpholine-*N*-oxide and after crystallization from ethanol afforded appropriate 2α,3α-dihydroxy-5-androstan-6-one derivatives (*II*). On treatment with trifluoroperoxyacetic acid in dichlormethane (Baeyer-Villiger oxidation), directly without



R = (a) H, (b) COC_3H_7, (c) COC_3F_7, (d) $COC_{11}H_{23}$

protection of 2α,3α-dihydroxy group, two lactones were obtained, mainly 6-oxo-7-oxa one (*IV*). The activity of obtained compounds (*II*, *III* and *IV*) in the bean second internode bioassay will be discussed.

This work was supported by grants Nos. 203/01/0083, A 4055204 and Z 4055905.

REFERENCES

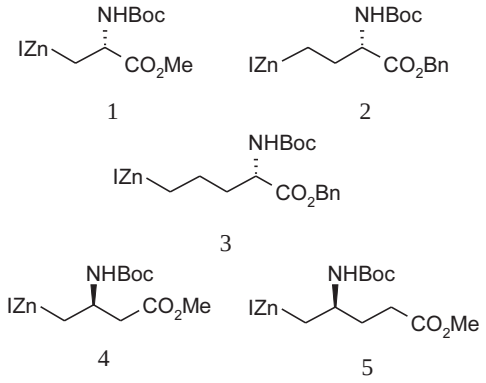
1. Strnad M., Kohout L.: Plant Growth Regul. *40*, 39 (2003).

RECENT ADVANCES IN THE USE OF ORGANOMETALLIC CHEMISTRY FOR THE SYNTHESIS OF AMINO ACIDS

RICHARD F. W. JACKSON

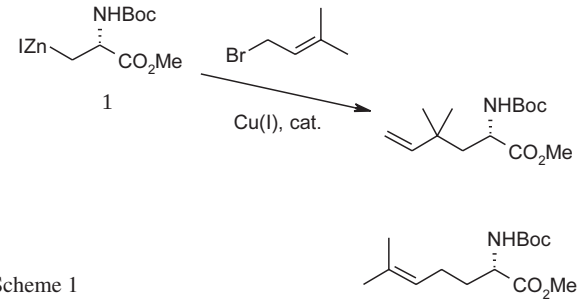
Department of Chemistry, University of Sheffield, Dainton Building, Brook Hill, Sheffield, S3 7HF, United Kingdom
E-mail: r.f.w.jackson@shef.ac.uk

The application of organometallic chemistry to the synthesis of highly functionalised target compounds has seen very significant advances in the last 15 years. In particular, the realisation that carbon-zinc bonds are compatible with many functional groups has transformed the general perception of the utility of organozinc compounds. This lecture describes the development of synthetic methods for the preparation of a wide variety of unnatural amino acids from simple, readily available amino acids[1], focussing on our recent work. The general approach that we have taken involves the conversion of the side-chain of naturally occurring amino acid derivatives, such as serine, aspartic acid and glutamic acid, into the corres-

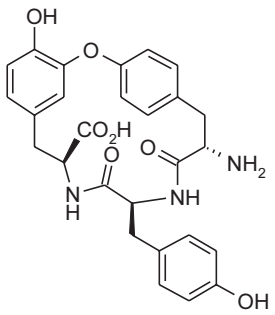


ponding primary alkyl iodide, followed by conversion into the organozinc reagents, exemplified by the structures 1 to 5. Subsequent palladium or copper catalysed reactions of these reagents allows the synthesis of a wide variety of functionalised amino acid derivatives, without loss of stereochemical integrity.

Applications to the synthesis of terpene/amino acid conjugates will be presented (Scheme 1)[2], which employs catalytic amounts of Cu(I). The compatibility of the carbon-zinc bond with highly functionalised compounds will be illustrated by the synthesis of macrocyclic tripeptides, specifically K13, in which the key step is an intramolecular carbon-carbon bond forming reaction[3].



Scheme 1



K-13

Finally, insights gained from spectroscopic and kinetic studies into the stability and reactivity of functionalised organozinc reagents[4,5], especially those bearing a β-amido group, will be presented. These have allowed the development of new reagents, with surprising stability.

REFERENCES

1. Gair S., Jackson R. F. W.: Curr. Org. Chem. *2*, 527 (1998).
2. Deboves H. J. C., Grabowska U., Rizzo, A., Jackson R. F. W.: J. Chem. Soc., Perkin Trans. 1 *2000*, 4284.
3. Perez-Gonzalez M., Jackson R. F. W.: Chem. Commun. *2000*, 2423.
4. Dexter C. S., Hunter C., Jackson R. F. W.: J. Org. Chem. *65*, 7417 (2000).
5. Jackson R. F. W., Rilatt I., Murray P. J.: Chem. Commun. *2003*, 1242.

LEAD TETRAACETATE-IODINE OXIDATION OF 23-SPIROSTANOLS

IZABELLA JASTRZĘBSKA, JACEK W. MORZYCKI*, URSZULA TROCHIMOWICZ

Institute of Chemistry, University of Bialystok, al. Pilsudskiego 11/4, 15-443 Bialystok, Poland
E-mail: morzycki@uwb.edu.pl

The reactions of 23-spirostanols with lead tetraacetate-iodine were studied under various conditions (different solvents, reaction time and temperature). It was found that both 23-spirostanols with an axial (23*R*) or equatorial (23*S*) hydroxy group afforded similar set of products irrespectively of configuration at C25. Presumably, the alkoxy radicals formed from the epimeric 23-spirostanols equilibrate *via* an open chain form.

The major product of reactions carried out at low temperatures was lactone obtained by loss of a C_5 fragment. Two minor products, the 17-iodo dialdehydes of opposite configuration at C17, accompanied the lactone.

The reactions performed at higher temperatures afforded mainly 20β-chlorolactones. They were accompanied by the

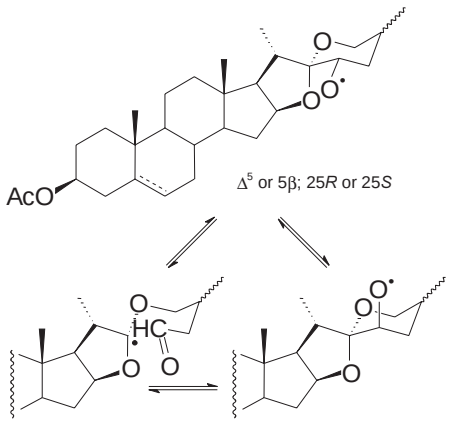


Fig. 1.

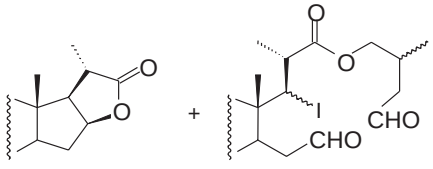


Fig. 2.

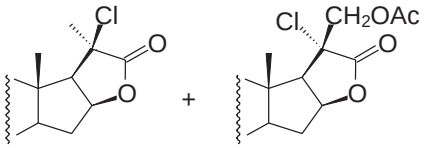


Fig. 3.

21-acetoxy derivative, but in the case of (25*R*)-23-spirostanols only. This product was formed as a result of remote intramolecular free medical functionalization of C21 methyl group followed by further oxidation.

No products of functionalization of a 25-methyl group were found among the reaction products. The mechanism of these reactions will be discussed.

REARRANGEMENTS OF 23-SPIROSTANOL AND 23-SPIROSTANONE DERIVATIVES

IZABELLA JASTRZĘBSKA, JACEK W. MORZYCKI *

University of Bialystok, Institute of Chemistry, al. Pilsudskiego 11/4, 15-443 Bialystok, Poland
E-mail: morzycki@uwb.edu.pl

Steroidal sapogenins bearing a good leaving group at C23 undergo a completely stereospecific rearrangement under a variety of conditions *via* a mechanism involving neighboring-group participation by the spiroketal oxygen atom in the departure of the nucleofuge from C23 (ref.[1]). The reactions of equatorial (23*S*)-23-bromo- or (23*S*)-23-tosyloxy-spirostanes with either the α (25*R*) or β (25*S*) oriented 25-methyl group lead to the bistetrahydrofuran products with inversion of configuration at C23. The product structures were confirmed by the X-ray and spectroscopic studies.

The reactions of the starting compounds with axial substituents (23*R*) at C23 require drastic conditions and result in the formation of the corresponding olefin accompanied by the rearranged product (in the case of the 25*S* isomer only).

23-Spirostanone derivative when treated with boron trifluoride undergoes rearrangement to an isomeric spiroketal

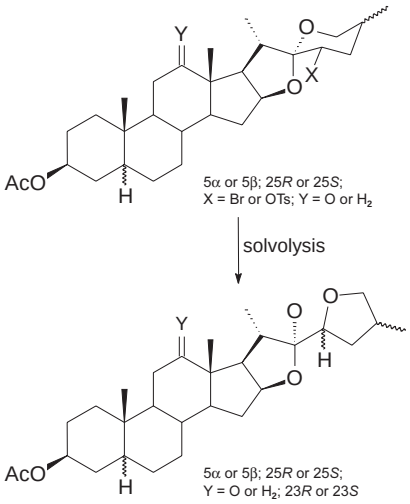


Fig. 1.

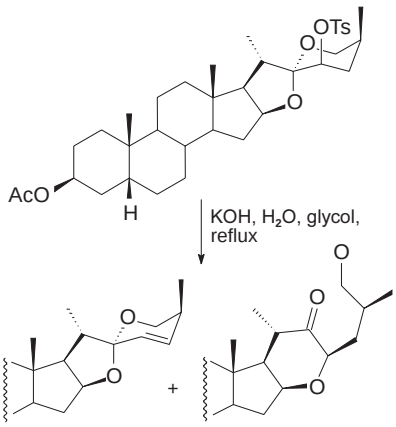


Fig. 2.

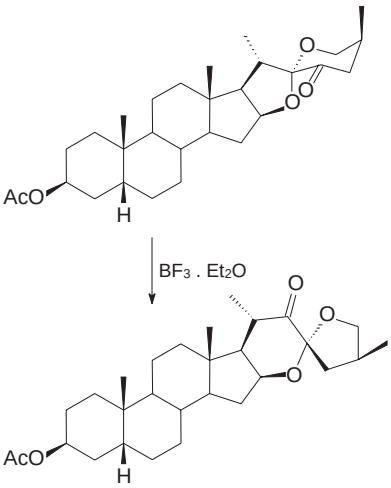


Fig. 3.

with the 22-oxo group. Its structure was confirmed by the X-ray studies.

REFERENCES

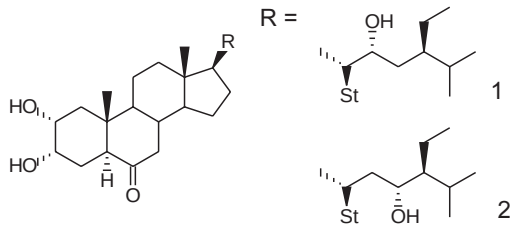
1. Anulewi Ostrowska R., Jastrzębska I., Morzycki J. W., Wójcik J.: J. Org. Chem. *67*, 6916 (2002).

SYNTHESIS OF NEW BRASSINOSTEROID ANALOGS HAVING ONLY ONE HYDROXYL GROUP AT THE SIDE CHAIN TO ASSESS THE IMPORTANCE OF THESE FUNCTIONALITIES

IBAN JOVÉ, XEVI HERNÀNDEZ, MARC AMORÓS, CARME BROSA *

Institut Químic de Sarrià, C.E.T.S., Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain
E-mail: brosa@iqs.url.es

Brassinosteroids (BRs) are recognised as the sixth class of plant hormones, in addition to auxins, gibberellins, cytokinins, abcisic acid and ethylene. They are steroidal phytohormones



that show remarkable plant physiological activities. BRs induce cell elongation and cell division, increase DNA and RNA polymerase activity, stimulate ethylene production, increase tolerance to stress due to temperature, water or salinity, act to protect against pesticides and increase crop yields[1].

The advantage of BRs from the other phytohormones is that they are active at very low concentrations (0.01–0.0001 ppm), about 100 times lower concentration than other plant hormones. So, the effects of BRs and the fact that are natural products, make quite good candidates for applications in agriculture.

Dealing with brassinosteroids, the knowledge of the minimum structural requirements needed for expressing activity is still a challenge. This not only will help to reduce synthetic efforts of new active BRs analogs but also to better understand their mode of action in plants. To achieve this goal in the most efficient way, a quantitative structure-activity relationship (QSAR) based on molecular modeling techniques has been developed in our group[2].

Following this model, we have found that the electrostatic charges play an important role in explaining the activity and that the hydrogen bonding could be one of the types of interaction that could take place on binding.

A comparative computational study of about 20 brassinosteroids analogs[2] and the antagonist KM-01 suggests that the region near to 23*R*-OH group of brasssinolide and the one near to 3α-OH group are more important for eliciting activity than the regions near C22 and C2 hydroxyl groups.

Attending to the importance of hydroxilic functionalities at the brassinosteroids side chain, our interest is focused in determining their contribution to the activity. With this aim, the synthesis of new brassinosteroid analogs having only one hydroxyl group at the side chain is required.

In this communication, we will present the synthetic strategy developed to obtain 1 and 2 as well as the bioactivity evaluated in the rice lamina inclination test. Moreover, we will discuss how the functionalities present at C22 and C23 can contribute to the activity.

This work was supported by a grant from Generalitat de Catalunya (No. 2001FI 00540). I. J. is grateful to Generalitat de Catalunya for a fellowship.

REFERENCES

1. Khripach V. A., Zhabinskii V. N., de Groot A. E.: *Brassinosteroids: A New Class of Plant Hormones*. Academic Press California, 1999.
2. Brosa C.: *Structure-activity Relationship*, in: *Brassinosteroids: Steroidal Plant Hormones* (Sakurai A., Yokota T., Clouse S., ed.), Chap. 9. Springer-Verlag, Tokyo 1999.

ENANTIOMERIC COMPOSITION OF GERMACRENE D IN *Aesculus hippocastanum*

BLANKA KALINOVÁ[a], ANNA-KARIN BORG-KARLSON[b], ALEŠ SVATOŠ[c]

[a]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic,* [b]*Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stocholm, Sweden,* [c]*Max Planck Institute, Winzerlaer Straße 10, D-07745 Jena, Germany*
E-mail: blanka@uochb.cas.cz

Sesquiterpene germacrene D is a chiral compound synthesized as one or both enantiomers in various plant, fungi and animals. It is an important intermediate in the formation of many sesquiterpenes *via* cyclization of farnesyl diphosphate catalysed by enantioselective synthases[1]. In higher plants the (–)-configuration of germacrene is most common[2], but in some species both enantiomers were found[3–5].

The horse chestnut tree, *Aesculus hippocastanum* (Hipocastanaceae), is a host for horse chestnut leafminer, *Cameraria ohridella*, a tiny moth spreading invasively within Europe. Chestnut trees release complex mixtures of volatiles attracting gravid females to oviposit. In the search for the biologically relevant plant attractants, we employed the gas chromatography linked with registration of female olfactory activity (female antennae, connected between two microelectrodes, were allowed to respond to GC eluate – GC-EAD). GC-EAD experiments and GC-MS analysis of *A. hippocastanum* head space samples (50 μg charcoal filters, 24 hr, air flow <500 ml.min^{-1}, double extraction with 40 ml of hexane) and SPME showed the presence of several active compounds including germacrene D.

The enantiomeric composition of germacrene was determined using two-dimensional GC system with DB-WAX and permethylated β-cyclodextrin capillary columns. In principle, Carbowax type column pre-separated the volatile mixture. When the compound of interest was about to elute, a micro--valve directed the compound to the chiral column (and back when the compound eluted). (+) and (–) enantiomeric pair of germacrene D was obtained by 30 min hexane extraction of *Solidago canadensis* L. (Asteraceae). The retention time of germacrene D from *A. hippocastanum* matched with retention times of (–) enantiomer. The correspondence of retention times was confirmed by co-injection of *S. canadensis* and *A. hippocastanum* extracts.

REFERENCES

1. Büllow N., König W. A.: Phytochemistry *55*, 141 (2000).
2. Stranden M., Borg-Karlson A.-K., Mustaparta H.: Chem. Senses *27*, 143 (2002).
3. Niwa N., Iguchi M., Yamamura S.: Chem Pharm. Bull. *28*, 997 (1980).
4. Schmidt C. O., Bouwmeester H. J., de Kraker J. W.,

König W. A.: Angew. Chem., Int. Ed. Engl. *37*, 1400 (1998).
5. Schmidt C. O., Bouwmeester H. J., Francke S., König W. A.: Chirality *11*, 353 (1999).

AFFINITY CHROMATOGRAPHY OF STEROL-BINDING PROTEINS ON COLUMNS WITH IMMOBILISED BRASSINOSTEROIDS, PLANT HORMONES WITH ANTI-STRESS ACTIVITY

MAREK KAMLAR[a,b], TOMÁŠ MACEK[a*], CSABA KONCZ[c], LADISLAV KOHOUT[a]

[a]Department of Natural Products, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, [b]Department of Biochemistry and Microbiology, ICT Prague, Technická 3, 166 28 Prague 6, Czech Republic, [c]Max-Planck-Institut fuer Zuechtungsforschung, Carl-von-Linne Weg 15, 50829 Koeln, Germany
E-mail: tom.macek@uochb.cas.cz, malakin@seznam.cz

Brassinosteroids (BRs) are polyhydroxysteroid phytohormones affecting diferentiation of plants, their growth, fertility, photomorphogenesis, seed germination, but also senescence or stress tolerance[1,2]. Mechanism of action of BRs is unclear. Probably it is mediated by membrane receptors, but certain role have also sterol-binding proteins and some other compounds[3,4].

The aim of this work was using affinity chromatography for isolation of proteins binding to brassinosteroids. Because it is not known which part of steroid molecule is responsible for interaction with binding proteins, three types of structure analogues of naturally occurring plant steroids with differently modified skeleton were used as ligands for binding to resin carrier. Columns were prepared with immobilised (20*S*)-2α,3α-dihydroxy-7-oxa-B-homo-5α-pregnan-6-one-20-carboxylic acid, carboxymethoxyoxime (22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-5α-cholestan-6-one and with 24-epibrassinolide[5,6]. As polymeric matrices for binding of BRs we tested: (*a*) resin with free aminogroups to form amide bond with -COOH group of modified BRs (agarose resin with adipic acid dihydrazide, or amino-PEGA with ethyleneglycol spacer), and (*b*) for the method using active esters exploited routinely in solid phase peptide synthesis a resin able to form esteric bond with -OH groups of BR molecule (NovaSyn® TG carboxyresin).

The plant extracts were obtained by grinding frozen leaves from *Nicotiana tabacum*. After homogenisation and centrifugation salts were removed from solution by gel filtration and the extract was applied to the bioaffinity matrix. Eluates were collected and after concentration step tested by SDS electrophoresis. The effectivity of columns was compared by the amount of protein bound and selectively eluted as seen on elfo.

The eluate yielded among others a protein of the same size from columns B and C, obtained from cytosolic fraction of tobacco callus, apparently produced under stress caused by the presence of sucrose. The analysis of primary structure has revealed, that the protein sequencing from N-end characterised the most abundant band as: SGVFEVHNNCPYTVWAAA, exhibiting 100 % homology with part of the sequence of the so called osmotin-like protein precursor, found in tobacco, which is known as compound synthesised in tobacco under stress conditions, especially after pathogen attack on the plant[7,8].

Osmotin-like protein precursor (OLPA)
– extracellular protein
– rel. mol. weight 27 652 Da, 251 AA
– belongs to PR (pathogenesis related) proteins
– has connection with plant adaptation to abiotic and biotic stress (caused by drying or other factors, pathogens like *Candida albicans*, *Trichoderma reesei* etc.)

Any connection of this protein with brassinosteroide hormone signal transduction was yet unknown. We are now investigating the effects of jasmonate, salicylic acid, ethylene etc., on levels of OLPA, and experiments with the expression of OLPA in *E. coli* are under way.

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

REFERENCES

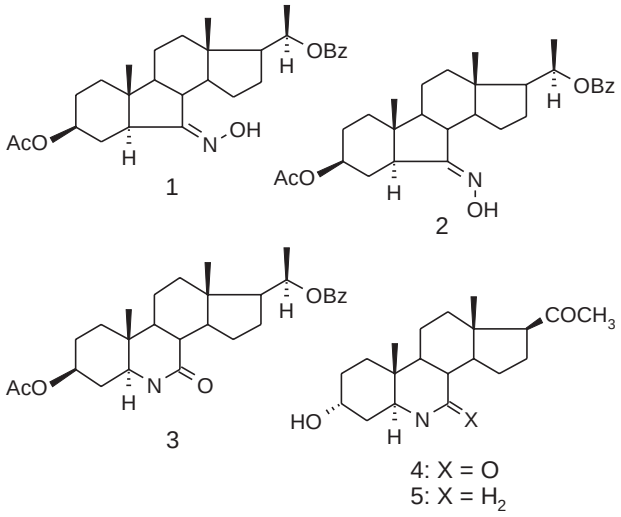
1. Clouse S. D., Langford M., McMorris T. C.: Plant Physiol. *111*, 671 (1996).
2. Szekeres M., Nemeth K., Kon Kalman Z., Mathur J., Kauschmann A., Altmann T., Redei G. P., Nagy F., Schell J., Koncz C.: Cell *85*, 171 (1996).
3. (a) Stokes T.: Trends Plant Sci. *9*, 366 (2000); (b) Mussig C., Altmann T.: Trends Endocrinol. Metab. *12*, 398 (2001).
4. Kohout L.: Collect. Czech Chem. Commun. *59*, 457 (1994).
5. Kohout L., Kasal A., Strnad M.: Collect. Czech. Chem. Commun. *61*, 930 (1996).
6. Liu D., Rhodes D., Paino D'Urzo M., Xu Y., Narasimhan M. L., Hasegawa P. M., Bressan R. A., Abad L.: Plant Sci. *121*, 123 (1996).
7. Abad L., D'Urzo M. P., Liu D., Narasimhan M. L., Reuveni M., Zhu J. K., Niu X., Singh N. K., Hasegawa P. M., Bressan R. A.: Plant Sci. *118*, 11 (1996).
8. Shih C. T., Wu J., Jia S., Khan A. A., Ting K. H., Shih D. S.: Plant Sci. *160*, 817 (2001).

NEUROSTEROID ANALOGUES: 6-AZA-ALLOPREGNANOLONE

A. KASAL[a], L. MATYÁŠ[a,b], M. BUDĚŠÍNSKÝ[a], C. SUŇOL[c], Z. KRIŠTOFÍKOVÁ[a]

[a]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, [b]Institute of Chemical Technology, 166 28 Prague 6, Czech Republic, [c]Department of Neurochemistry, Institut d'Investigacions Biomédiques de Barcelona, 08036 Barcelona, Spain
E-mail: kasal@uochb.cas.cz

The introduction of a nitrogen atom into a molecule of allopregnanolone could increase the solubility of the products. This is why we decided to prepare aza-analogues of some



neurosteroids because the activity of some of our analogues was often marred by their low solubility in body liquids and the usual ways of making compounds more soluble did not help.

6-Azasteroid have been successfully prepared[1,2] from steroidal 5,6-seco acid, here we present an alternative synthesis based on the transformation of B-norsteroids. (20*R*)-6-Oxo-B--nor-5α-pregnane-3β,20-diyl 3-acetate 20-benzoate reacted with hydroxylamine to give two oximes 1 and 2 (6-oxosteroids of the normal series give one oxime only). The Beckmann rearrangement of the major oxime 1 yielded the expected lactam 3. Standard procedures were used to convert the latter into the title compound.

The biological activity of the compounds 4 and 5 will be determined *in vitro*, using labelled muscimol, TBPS and flunitrazepam as ligands.

The authors are indebted to Mrs. M. Sedláčková for technical help. This work was supported by grants No. S5011007 and Z4 055 905.

REFERENCES

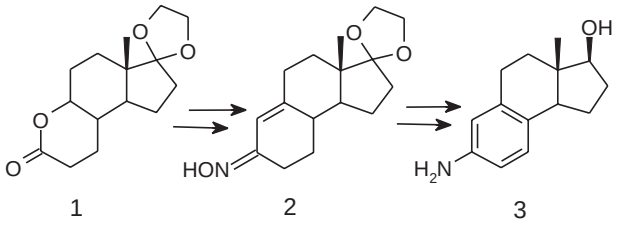
1. Morzycki J. W., Wilczewska A. Z.: Tetrahedron *52*, 14057 (1996).
2. Guarna A., Occhiato E. G., Danza G., Conti A., Serio M.: Steroids *63*, 355 (1998).

PREPARATIVE METHOD FOR CONVERSION SUBSTITUTED CYCLOHEXENONE INTO ANILINE DERIVATIVE

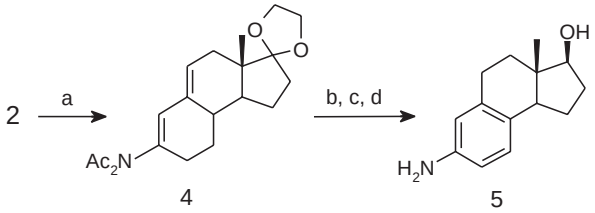
MARINUS B. GROEN*, ANNA I. KATSIATKINA

Department of Medicinal Chemistry, N.V. Organon, P.O. Box 20, 5340 BH Oss, The Netherlands
E-mail: Marinus.Groen@organon.com

A reliable procedure for converting tricyclic lactone 1 into aniline 3 through intermediate enoxime 2 was developed.

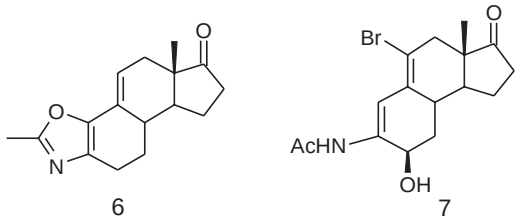


Scheme 1



Scheme 2

(a) Ac_2O, Py, Rf, 27 h; (b) $CuBr_2$, LiBr, CH_3CN, Rf, 2h; (c) $Ca(BH_4)_2$; (d) N_2H_4 H_2O



Scheme 3

The strategy included conversion of enoxime 2 in dienediacetamide 4 followed by aromatization using a bromination-dehydrobromination sequence.

Our investigations provided several other products as well, the formation of which depended on the reaction conditions.

Thus, oxazole 6 was obtained when conditions of the Semmler-Wolf reaction were applied to 2. Substituted dienacetamide 7 was formed as a by-product in the bromination-dehydrobromination of 4.

BRASSINOSTEROIDS: PRESENT STATUS AND NEW TRENDS OF RESEARCH AND APPLICATION

VLADIMIR A. KHRIPACH

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141, Minsk, Kuprevich Str. 5/2, Belarus

The discovery of brassinosteroids (BS) and their extensive investigations during last two decades brought a new understanding of the hormonal functions of steroids in living organisms, because in addition to the previously known role as hormones of animals and fungi their role of plant hormones had been recognized. The importance of the obtained results

is not bounded by pure basic knowledge. One of the key physiological characteristics of new plant hormones, when applied exogenously to growing plants, is their capability to stimulate plant growth and development. That is why their application in agriculture was considered to be promising from the beginning of BS study and nowadays found its realization.

Progress in brassinosteroid research has been extremely rapid. After publication of brassinolide, the first member of the series, less than twenty years were necessary to overcome the distance to practical use of BS in agriculture as crop-yield--increasing and plant-protecting agents. During this period a number of naturally occurring BS of different structure were identified and prepared synthetically, their physiological action investigated, and many problems connected with industrial-scale production and official status of new agrochemicals have been solved. Among them: development of economically reasonable synthetic methods of preparation of BS, field-scale biological trials with different crops, toxicological studies, etc. For none of the other plant hormones, although studied for a much longer time, there has been similar development.

The question "Are the BS real plant hormones, or not?", which was discussed in the beginning of BS-study, can be considered today as finally solved. All the data on properties of BS in plants, and discovery of genes that are specifically expressed by BS, and close approaching to the identification of BS-receptors make sure that BS are real plant hormones. BS show various kinds of regulatory effects in many fundamental processes of plant growth and development, such as cell enlargement and cell division, regulation of the gravitropic response, tissue differentiation, expression of photoregulated genes, reaction to abiotic and biotic stresses, and many others. BS-deficient mutants demonstrate abnormal development (dwarfism, defects in leaf anatomy, male sterility and others) that could be rescued by exogenously applied BS. These data, and many others collected by now, indicate an exclusive role of BS in all phases of plant development, from cellular level to developed organism and further generations.

The wide spectrum of BS-activities is the result of a cascade of biochemical shifts which is initiated *via* direct action of BS on the genome or by an extragenetic route. The first way *via* genome expression activates the biosynthesis of proteins (enzymes) and is responsible for the slow reactions of plants on exogenous hormone. The second one influences the membrane properties and is responsible for the quick reactions. Both routes assume the involvement of a system of secondary messengers and can act together with overlapping and close interconnections between them.

During recent years, the molecular genetic methodology and use of special mutants of Arabidopsis brought real break--through in the mechanistic studies of BS-action. The findings in disclosure of the mechanism stimulate further efforts directing to the localization of the effect of BS in the chain of signaling events in the plant cell. One of the indicators on the promising area is the data on the genetically-determined involvement of BS in the light-regulated plant development. Our recent study on the effect of BS on the hormonal balance in light-dependent development of wild type *Arabidopsis* and its mutants defective in genes encoding synthesis of some photoreceptors showed clear relationships between the responses mediated by these photoreceptors and action of BS. The results suggest an important function of BS in light and hormone signaling cross talk and give a new confirmation of their central role among other phytohormones.

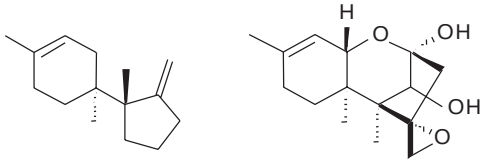
New data covering some synthetic, analytical and mechanistic aspects of BS will be discussed.

STUDIES ON THE SYNTHESIS OF CURVULAROL AND PHYTOSIDEROPHORES

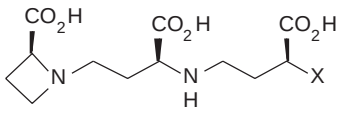
TAKESHI KITAHARA

Department of Applied Biological Chemistry, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
E-mail: atkita@mail.ecc.u-tokyo.ac.jp

Studies on the synthesis of the following natural products, a sesquiterpene, curvurarol 2, and phytosiderophores 3, 4 and related analogs with remarkable biological activities will be discussed.



trichodiene 1 curvularol 2



deoxymugineic acid (DMA, 4): X = OH
nicotianamine (NA, 5): X = NH_2

Fig. 1. Target molecules

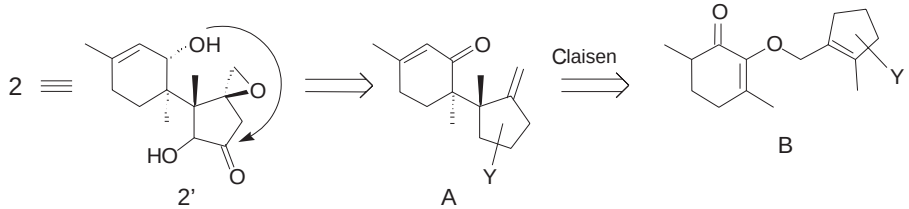


Fig. 2. Retrosynthesis of Curvularol 1

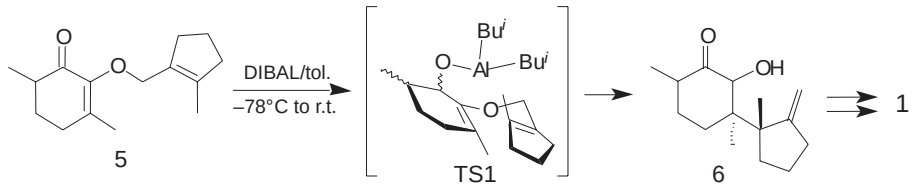


Fig. 3. Synthesis of Trichodiene 1

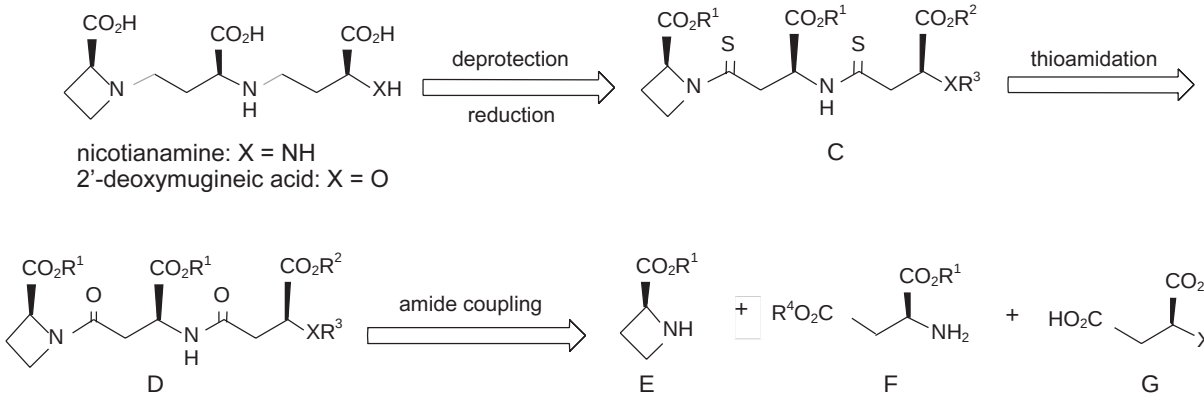


Fig. 4. Retrosynthesis of Phytosiderophores

Curvularol 2, a trichothecane sesquitepene, was isolated as a potent cell cycle inhibitor[1]. We decided to use Claisen rearrangement for its synthesis as shown in Fig. 2. In fact, we succeeded in the synthesis of trichodiene 1 with basic skeleton of 2 *via* aluminum-promoted process as shown in Fig. 3 (ref.[2]). Synthesis of curvularol 2 starting from a functionalized allylic alcohol is now in progress and details will be discussed precisely in the lecture.

Deoxymugineic acid 3, nicotianamine 4 and congeners are remarkable phytosiderophores which enable the Gramminaceous plants to survive under iron-deficient condition. We developed a simple, expeditious and efficient method to them in large quantity as shown in Fig. 4 (ref.[3]). We also obtained many related analogs *via* this route[4]. Through the biological studies using these phytosiderophores, we found extremely interesting results on their biological functions as phytosiderophores[5]. Details of the synthesis and of biological studies will be discussed in the lecture.

REFERENCES

1. Honda Y., Ueki M., Okada G., Onose R., Usami R., Horikoshi K., Osada H.: J. Antibiot. *54*, 10 (2001).
2. Watanabe H., Kawasaki T., Ishigami K., Kimura M., Yamaguchi I., Kitahara T.: submitted for publication.
3. Klair S. S., Mohan H. R., Kitahara T.: Tetrahedron Lett. *39*, 89 (1998).
4. Miyakoshi K., Oshita J., Kitahara T.: Tetrahedron *57*, 3355 (2001).
5. Oshita J., Miyakoshi K., Osawa H., Miyachi H., Kitahara T.: submitted for publication.

BILE ACIDS AS BUILDING BLOCKS OF SUPRAMOLECULAR AND NANO-SIZED HOSTS

ERKKI T. KOLEHMAINEN*, JARI TAMMINEN, ELINA VIRTANEN, ARTO VALKONEN, JUHA KOIVUKORPI

Department of Chemistry, University of Jyväskylä, P.O. Box 35, FIN-40014, University of Jyväskylä, Finland
E-mail: ekolehma@cc.jyu.fi

From the surface and colloid chemical point of views bile acid conjugates and bile salts have been a research object for decades[1]. Natural bile acid derivatives are detergent like molecules helping in the solubilization of dietary fats and lipophilic vitamins. Owing to their amphiphilic character bile acid

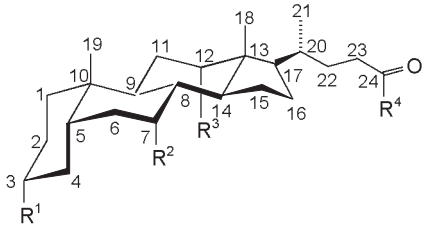


Fig. 1. Common human C_{24}-bile acids. $R^1 = R^2 = R^3 = H$, $R^4 = OH$ (5β-cholan-24-oic acid); $R^1 = R^4 = OH$, $R^2 = R^3 = H$ (3α-hydroxy-5β--cholan-24-oic acid = lithocholic acid); $R^1 = R^2 = R^4 = OH$, $R^3 = H$ (3α,7α-dihydroxy-5β-cholan-24-oic acid = chenodeoxycholic acid); $R^1 = R^3 = R^4 = OH$, $R^2 = H$ (3α,12α-dihydroxy-5β-cholan-24-oic acid = deoxycholic acid); $R^1 = R^2 = R^3 = R^4 = OH$ (3α,7α,12αα-trihydroxy-5β-cholan-24-oic acid = cholic acid)

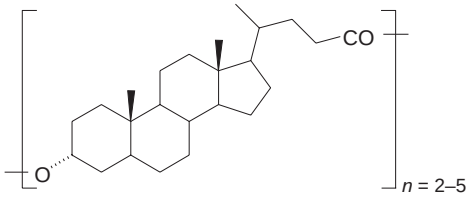


Fig. 2. Structures of dimeric to pentameric cyclolithocholates

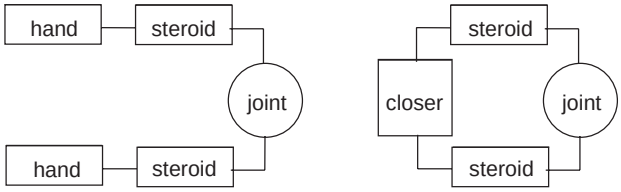


Fig. 3. General structures of bile acid clefts (left) and cholaphanes where closer contains some aryl group (right). The joint can be ethylene glycol or pyridine dimethanol diester or piperazine diamide, hands aromatic or polyaromatic carboxylic acid esters and the closers aromatic dicarboxylic acid esters

conjugates and bile salts tend to self assembly or form aggregates, which can incorporate also xenobiotics, such as aromatic hydrocarbons in their micelles[2].

A renaissance of bile acid research is essentially associated with the advances of supramolecular chemistry[3–9]. Reasons for that are in the structures of bile acids (Fig. 1), which are multifunctional molecules offering a plethora of structural modifications. In addition there exists a large variety of bile acid derivatives characterized by their ^{13}C NMR data[10] helping significantly the structural elucidation of novel bile acid derivatives.

At first our interest focused on the syntheses of cyclolithocholates (Fig. 2), which are head-to-tail macrolides of bile acids[11]. However, more structural variation is achieved by introducing other linking groups or spacers and ring closing moieties in these structures. Typically such groups are ethylene glycol[12], linear diamines[13], piperazine[14–16], dicarboxylic acids such as phthalic acid and its isomers[12,14], isomeric bipyridine and pyridine dicarboxylic acids[15,17], five membered heterocycles such as thiophene[16] and pyrrole dicarboxylic acid[17], pyridine dimethanol[17] etc. General structures of these kinds of molecular clefts or cholaphanes are described in Fig. 3.

In addition, we have determined X-ray crystal structures for some poorly crystallizable bile acid derivatives (Fig. 4), which form a basis for comparison of experimental and quantum chemically calculated (*ab initio*/HF) structures[18,19]. Depending on the properties of the joints and closers and whether the structure is open or closed these molecules show different flexibilities and dynamic behavior. For example, we found that some ^{13}C NMR chemical shifts of cyclopentane D ring and *iso*-pentanoic side chain in bile acid fragment are sensitive to the steric strain, which is related to the size of the macrocycle.

Further, experimental and density functional theoretical (DFT) ^{13}C and ^{17}O NMR chemical shifts[18,19] of various monomeric bile acid derivatives showed excellent agreements, which can be further used in analyzing and modeling more complex structures.

Our most recent interest are focused on syntheses of bile acid conjugates with linear aminoalcohol homologues[17], which show some gelating properties in organic solvents as well as various Schiff bases formed from carbonyl bearing bile acid derivatives (oxidation products of bile acids) and cyclic and linear amines. Further, syntheses of dendrimers[20] and resorcinarenes[17] with bile acid derived structural fragments are under progress.

The aim for preparations of various bile acid derivatives is to find suitable structures for selective molecular recognition, acting as prodrugs or drug carriers for organ (especially liver) specific targeting, novel organogelators or liquid crystalline phases *e.g.* Our recently upgraded MS laboratory also offer improved potential to perform competitive binding studies for estimating quantitative recognition properties of the synthesized novel structures by ESI/MALDI-TOF methods.

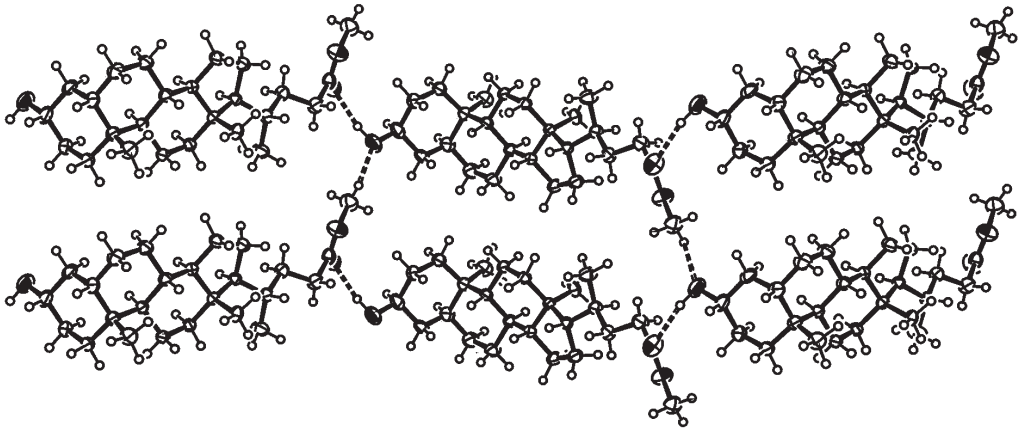


Fig. 4. Crystal packing of methyl lithocholate showing head-to-tail intermolecular hydrogen bonding between C_{24} = O…H-O-C_3 and one proton of the ester methyl CH_3…O-C_3 with the other molecule in the adjacent polymeric steroidal chain

In conclusion, bile acids although studied for decades still offer new potential and challenges for chemists. The medical, pharmaceutical, biochemical, organic, supra-molecule and nano-chemical and many other application possibilities of novel bile acid derivatives are almost unlimited.

REFERENCES

1. Kritchevsky D., Nair P. P., in: *The Bile Acids: Chemistry, Physiology and Metabolism* (Nair P. P., Kritchevsky D., ed.), 1, 3. Plenum, New York 1971.
2. Kolehmainen E.: *Doctoral Thesis*. Department of Chemistry, University of Kuopio, Publications of the University of Kuopio, Natural Sciences, Original Reports 1/ 1990, Finland (1990).
3. Davis A. P.: Chem. Soc. Rev. *22*, 243 (1993).
4. Li Y., Dias J. R.: Chem. Rev. *97*, 283 (1997).
5. Walliman P., Marti T., Fürer A., Diederich F.: Chem. Rev. *97*, 1567 (1997).
6. Davis A. P., Bonar-Law R. P., Sanders, J. K. M., in: *Comprehensive Supramolecular Chemistry* (Atwood J. L., Davies J. E. D., McNicol D. D., Vögtle F., ed.), 4, 257. Elsevier, Oxford 1996.
7. Miyata M., Sada K., in: *Comprehensive Supramolecular Chemistry* (Atwood J. L., Davies J. E. D., McNicol D. D., Vögtle F., ed.), 6, 147. Elsevier, Oxford 1996.
8. Tamminen J.: *Doctoral Thesis*. Department of Chemistry, University of Jyväskylä, Research Report No. 78, Jyväskylä, Finland (2000).
9. Tamminen J., Kolehmainen E.: Molecules *6*, 21 (2001).
10. Dias J. R., Gao H., Kolehmainen E.: Spectrochim. Acta, Part A *56*, 53 (2000).
11. (a) Lappalainen K.: *Doctoral Thesis*. Department of Chemistry, University of Jyväskylä, Research Report No. 62, Jyväskylä, Finland (1997); (b) Lappalainen K., Kolehmainen E., Kotoneva J.: Magn. Reson. Chem. *34*, 316 (1996).
12. (a) Kolehmainen E., Tamminen J., Lappalainen K., Torkkel T., Seppälä R.: Synthesis *1996*, 1082; (b) Tamminen J., Kolehmainen E., Laihia K., Mänttäri P., Salo H.: Magn. Reson. Chem. *37*, 163 (1999); (c) Kolehmainen E., Tamminen J., Kauppinen R., Linnanto J.: J. Inclusion Phenom. Macrocyclic Chem. *35*, 75 (1999); (d) Tamminen J., Kolehmainen E., Linnanto J., Salo H., Mänttäri P.: Magn. Reson. Chem. *38*, 877 (2000).
13. Tamminen J., Kolehmainen E., Linnanto J., Vainiotalo P., Vuorikoski S., Kauppinen R.: J. Inclusion Phenom. Macrocyclic Chem. *37*, 121 (2000).
14. Tamminen J., Kolehmainen E., Haapala M., Linnanto J.: Synthesis *2000*, 1464.
15. Virtanen E., Tamminen J., Haapala M., Mänttäri P., Kauppinen R., Kolehmainen E.: Magn. Reson. Chem., in press.
16. Virtanen E., Koivukorpi J., Tamminen J., Mänttäri P., Kolehmainen E.: J. Organomet. Chem. *668*, 43 (2003).
17. Virtanen E., Tamminen J., Kärkkäinen K., Kauppinen R., Kolehmainen E.: unpublished results.
18. Virtanen E., Nissinen M., Suontamo R., Tamminen J., Kolehmainen E.: J. Mol. Struct. *649*, 207 (2003).
19. Virtanen E., Valkonen A., Tamminen, J., Kolehmainen E.: J. Mol. Struct. *650*, 201 (2003).
20. Ropponen J., Tamminen J., Kolehmainen E., Rissanen K.: Synthesis, submitted.

COMPARATIVE STUDY ON CAMPESTEROL SYNTHESIS

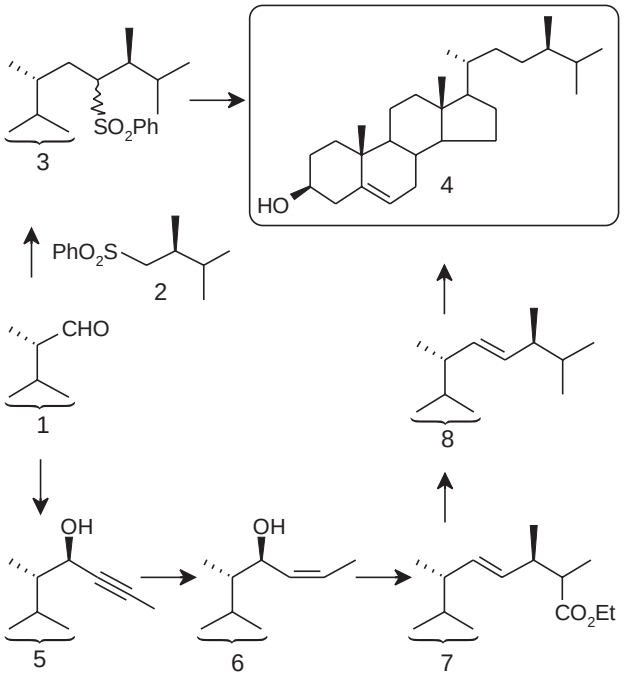
OLGA V. KONSTANTINOVA, VLADIMIR A. KHRIPACH, VLADIMIR N. ZHABINSKII, ALEXEY V. ANTONCHICK, ANDREY P. ANTONCHICK, NATALYA B. KHRIPACH

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich Str. 5/2, Minsk, 220141 Belarus
E-mail: vz@iboch.bas-net.by

Campesterol 4 is one of the basic components of plant cell membranes and an important biosynthetic precursor of a number of plant steroids, e.i. brassinosteroids[1]. In spite of the abundant distribution of campesterol 4, its isolation from plants in pure state is a rather complicated task, because of the presence of other sterols with very similar structure, such as sitosterol and 22-dihydrobrassicosterol. The only way to have pure campesterol 4 for biological studies is chemical synthesis.

Two synthetic schemes from aldehyde 1 to campesterol 4 have been investigated. Addition of lithium methylacetylene to aldehyde 1 followed by hydrogenation of propargyl alcohol 5 on Lindlar catalyst and Claisen rearrangement of allyl alcohol 6 gave ester 7. The last compound was transformed to campesterol 4 through intermediate 8. The drawback of this approach was partial epimerisation of C-24 during hydrogenation of intermediate 8 (ref.[2]).

The alternative way to campesterol 4 included addition of fragment 2 to aldehyde 1 and final removal of the phenylsulfoxide group.



Supported by the Belorussian Foundation for Fundamental Research (grant N X01MC-001).

REFERENCES

1. Khripach V. A., Zhabinskii V. N., de Groot A.: *Brassinosteroids: A New Class of Plant Hormones*. Academic Press, San Diego 1999.
2. Khripach V. A., Zhabinskii V. N., Konstantinova O. V., Khripach N. B., Antonchick A. P., Schneider B.: Steroids *67*, 597 (2002).

ECDYSTEROIDS AND RELATED MOLECULES IN ANIMALS AND PLANTS

RENÉ LAFONT

Université Pierre et Marie Curie, Institut de Biologie Intégrative, Laboratoire d'Endocrinologie Moléculaire et Evolution, 7 Quai Saint Bernard, Case 29, 75252 Paris, France
E-mail: rene.lafont@snv.jussieu.fr

Ecdysteroids are polyhydroxylated steroids firstly isolated from insects (1954) and later discovered in plants (1966). This family of molecules bears unique structural features (a *cis*-A/B ring junction, a 7-en-6-one and a 14α-OH) and it comprises more than 300 representatives[1].

Zooecdysteroids are present in all Arthropods and represent "moulting hormones" which control in fact not only their growth (i.e. moults and metamorphosis) but also their reproduction. If 20-hydroxyecdysone is the most common ecdysteroid, some diversity is observed within arthropod ecdysteroids as concerns their number of carbon atoms (27 to 29) and the number and position of hydroxyl groups. The biosynthetic pathway has not yet been fully elucidated, and a "black box" remains concerning the early steps, but new approaches have recently allowed the full characterization of several biosynthetic enzymes belonging to the cytochrome P450 (CYP) family thanks to *Drosophila* developmental (*halloween*) mutants[2,3].

Besides their classical endocrine roles, ecdysteroids may fulfill allelochemical functions in some Arthropods, and this can be examplified by the marine Arachnid (*Pycnogonum litorale*) which accumulates huge amounts of ecdysteroids in its integument as a defense mechanism against attack by crabs[4], and the Chrysomelid beetle *Chrysolina carnifex*, which incorporates large amounts of ecdysteroids in its defensive secretions[5].

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

Primitive Invertebrates (e.g. Sponges) contain a wide array of polyhydroxylated steroids, including true ecdysteroids, the origin of which need to be determined[6]. Whether some of these polyhydroxylated steroids are biogenetically related to ecdysteroids (= proto-ecdysteroids?) and how the Arthropod ecdysteroids have appeared is an important open question. Molecular approaches on biosynthetic enzymes and/or receptors will perhaps provide some answers in a near future.

Phytoecdysteroids have been found in ca. 6 % of the analyzed plant species (Ferns, Gymnosperms, Angiosperms) and in a few species of fungi; ecdysteroid-related molecules (pinnatasterols) are present in some Algae too[7,8]. Although structurally related to brassinosteroids, ecdysteroids have no established physiological role in plants, and they represent secondary metabolites able to protect plants against phytophagous Insects (and soil Nematodes?) through toxic and/or deterrent effects[8,9].

The diversity of phytoecdysteroids is much higher than that of zooecdysteroids, but this could be a simple consequence of their high levels in plants (their concentrations may reach 30 $g.kg^{-1}$ dry weight), which allows their more convenient isolation. A single plant species contains in fact a complex ecdysteroid cocktail[10]. Their biosynthetic pathway is not better known than that of Insects, but from an evolutionary point of view it would be of great interest to elucidate it and thus to determine whether both proceed through the same steps. Ecdysteroid distribution within plant organs does not follow a common pattern – they accumulate mainly in underground or aerial parts, in stem bark, flowers and/or seeds, … – and within a given specimen this pattern may change during ontogeny, which raises fundamental questions concerning the site(s) of production and the transport systems within the plant. These questions will be illustrated with the spinach *Spinacia oleracea*[11].

Such a survey would not be complete without describing the pharmacological effects of ecdysteroids on Mammals and Humans. Soon after the discovery of phytoecdysteroids, and probably in connection with the idea of using them as pesticides, ecdysteroid effects were tested on Mammals (*in vivo* and *in vitro*). These studies showed, besides a very low toxicity (LD50 >6 $g.kg^{-1}$ body weight in mice), a wide array of pharmacological actions[12], among which we will essentially comment the anabolic and hypoglycemic ones. Interestingly, several plants used in traditional medicine belong to the ecdysteroid-rich species (e.g. *Leuzea carhamoides*, *Pfaffia paniculata*, *Ajuga iva*, …), and their effects could be explained (at least in part) by their high ecdysteroid content.

Finally, what are the practical uses of ecdysteroids? A first use connected with their physiological role in Insects concerns the improvement of silk production[13] and of honeybee health[14]. A second use concerns the development of inducible gene expression systems, both *in vitro* with cell cultures (i.e. for fundamental research on gene function) and *in vivo* (with the aim of developing gene therapy systems)[12]. A third use is related with the above-described anabolic effects, and indeed there is a tremendously developing offer on the web of ecdysteroid-containing preparations for sportsmen and bodybuilders[12,15]. Natural ecdysteroids have no development in agriculture at the moment, however synthetic non-steroidal ecdysteroid agonists prove efficient in the field[16]; a better understanding of ecdysteroid biosynthesis and of its regulation will possibly allow new strategies for crop auto-protection to be developed in the future.

REFERENCES

1. Lafont R., Harmatha J., Marion-Poll F., Dinan L. N.,

Wilson I. D.: *Ecdybase* (2002); http://ecdybase.org, 5/6 2003.
2. Gilbert L. I., Rybczynski R., Warren J. T.: Annu. Rev. Entomol. *47*, 883 (2002).
3. Warren J. T., Petryk A., Marqués G., Jarcho M., Parvy J.-P., Dauphin-Villemant C., O'Connor M. B., Gilbert L. I.: Proc. Natl. Acad. Sci. U.S.A. *99*, 11043 (2002).
4. Tomaschko K. H. : Arch. Insect Biochem. Physiol. *41*, 89 (1999).
5. Laurent P., Braekman J. C., Daloze D., Pasteels J. M.: Chemoecology (2003), in press.
6. Costantino V., Dell'Aversano C., Fattorusso E., Mangoni A.: Steroids *65*, 138 (2000).
7. Lafont R.: Rus. J. Plant Physiol. *45*, 276 (1998).
8. Dinan L.: Phytochemistry *57*, 325 (2001).
9. Marion-Poll F., Dinan L. N., Lafont R., in: *Biopesticides d'origine végétale* (Regnault-Roger C., Philogène B. J. R., Vincent C., ed.), p. 97. 2002.
10. Báthori M., Girault J.-P., Kalasz H., Mathé I., Dinan L. N., Lafont R.: Arch. Insect Biochem. Physiol. *41*, 1 (1999).
11. Schmelz E. A., Grebenok R. J., Ohnmeiss T. E., Bowers W. S.: Arch. Insect Biochem. Physiol. *51*, 204 (2002).
12. Lafont R., Dinan L.: *J. Insect Science 3:7* (2003); http://www.insectscience.org/3.7, 9/6 2003.
13. (a) Chandrakala M. V., Maribashetty V. G., Jyothi H. K.: Curr. Sci. *74*, 341 (1998); (b) Ninagi O., Maruyama M.: Jpn. Agric. Res. Quarterly *30*, 123 (1996).
14. Kholodova Yu. D.: Ukr. Biokhim. Zh. *73*, 21 (2001).
15. Báthori M.: Mini Rev. Med. Chem. *2*, 285 (2002).
16. Dhadialla T. S., Carlson G. R., Le D. P.: Annu. Rev. Entomol. *43*, 545 (1998).

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

HENRY LARDY, ASHOK MARWAH, PADMA MARWAH, NANCY KNEER

Enzyme Institute, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin, USA

The C_{19} steroids are derived from cholesterol *via* pregnenolone to a main source, 3β-hydroxyandrost-5-en-17-one, commonly referred to as DHEA. This molecule was first identified by Butenandt and Dannenbaum[1] who isolated it from male human urine as the 3-chloro substituted steroid and recognized that the halogen had been introduced by treating a urine fraction with HCl. The chemical properties of DHEA have been, and continue to be, actively investigated by several eminent Czech scientists. The physiological actions of DHEA were pioneered by Dr. Jeri Sonka of Charles University who summarized ten years of his work in a useful monograph[2].

Because DHEA has many desirable physiological properties but displays these only weakly, we began to search for metabolites that might be more active than this parent steroid. There were many known metabolites to be examined and we developed an assay with which to measure their relative activities[3]. Like the thyroid hormone, DHEA induces the formation of liver mitochondrial glycerophosphate dehydrogenase (GPDH) and cytosolic malic enzyme (ME) when fed or injected into rats and this assay provides a semi-quantitative measure of one activity of these steroids.

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

DHEA incubated with rat liver homogenate fortified with ATP, NADPH and malate is converted to some 40 different steroids. Repeated sampling and analysis of products formed at short time intervals disclosed the conversion of DHEA to 7α-hydroxyDHEA, to 7-oxoDHEA, to 7β-hydroxyDHEA in sequence. Half of the DHEA accumulates as androst-5-ene-3β,17β-diol (Adiol) thus confirming findings of Schneider and Mason with rabbit liver[4]. DHEA is hydroxylated at position 7α by the known P450 7B. 7α-HydroxyDHEA is oxidized by 11β-hydroxy steroid dehydrogenase (flip orientation) to produce 7-oxoDHEA. 7-oxo-DHEA (in reverse orientation) is subject to reduction by the same enzyme to produce 7β-hydroxyDHEA. These steroids are subject to sulfation at position 3β and to reduction at position 17. Sulfation then also occurs at 17β. The ability to induce the formation of rat liver GPDH and cytosolic ME increases in the order of synthesis: DHEA < 7α-hydroxyDHEA < 7-oxoDHEA < 7β-hydroxyDHEA indicating they are on their way to become an active hormone. 7-OxoDHEA is far more effective than DHEA as an enhancer of memory in old mice. While these *in vitro* conversions are very rapid and involve relatively large quantities of steroids, we must recall that although DHEA is the most abundant steroid in human blood plasma, less than 1 % of the total circulates as the free steroid. And it is the free steroid that undergoes the metabolic transformations. The low concentration *in vivo* limits all of the conversions to other active molecules.

Like rat liver, isolated mouse adipocytes (3T3-L1) also convert DHEA to a variety of steroids; the yield of Adiol is 70 % of the added DHEA and it is excreted from the cells to the aqueous medium[5]. The formation of Adiol is important because Adiol possesses androgen activity that is not inhibited by hydroxyflutamide or bicalutamide, two agents that are commonly used to treat prostate cancer[6].

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

Thus it is possible that the reason growth of some prostate cancers becomes resistant to anti-androgen agents is that Adiol is the androgen that is stimulating their growth. Therefore it is important to find agents that inhibit the androgen activity of Adiol as well as of testosterone and dihydrotestosterone. Two steroids bearing ethynyl groups at position 17α have some ability to thwart the androgen activity of Adiol[7] and recent work has led to even more effective compounds. For example, 3β-acetoxyandrost-1,5-diene-17-ethylene ketal (ADEK) is an effective inhibitor of the androgenic activity of Adiol as well as that of dihydrotestosterone[8]. The agonist effect of ADEK is

less than that of hydroxyflutamide and therefore is less likely to induce withdrawal response in prostate cancer patients.

The metabolic conversion of steroids to more active structures beyond 7β-hydroxyDHEA has not yet been defined. There are several sulfated esters and glucuronides produced but the ones we have tested are not highly active. Hydroxylation at position 16a is especially prominent in children. 16α--HydroxyDHEA and 16-oxoDHEA are completely inactive in our rat assay but 3β,16α-Dihydroxyandrost-5-ene-7,17-dione and 3β,7β,16α-trihydroxyandrost-5-ene-17-one are as active as 7-oxoDHEA. Likewise, 3β,16α,17β-androstene-triol is inactive but introduction of an oxo group at position 7 restores activity. Thus it appears that if the active hormone produced from DHEA carries oxygen at position 16, that oxygen must be introduced after 7 is oxygenated. Expanded A or B ring derivatives of DHEA are inactive but 3β-acetoxy-17a-oxa-androst-5-ene-7,17-dione is fully active[9]. There are many products formed in liver tissue from DHEA that have not yet been completely characterized. They are the basis of our present work.

REFERENCES

1. Butenandt A.: Dannenbaum H.: Z. Physiol. Chem. *229*, 192 (1934).
2. Sonka J.: Acta Univ. Carol. Med. *71*, 171 (1976).
3. *Hormones, Thermogenesis and Obesity* (Lardy H., Stratman, ed.), pp. 415–426. Elsevier 1989.
4. Schneider J. J., Mason H. L.: J. Biol. Chem. *172*, 771 (1948).
5. Gomez F. E., Miyazaki M., Kim Y.-C., Marwah P., Lardy H. A., Ntambi J. M., Fox B. G.: Biochemistry *41*, 5473 (2002); Lardy H. A., et al.: manuscript in preparation.
6. Miyamoto H., Yeh S., Lardy H., Messing E., Chang C.: Proc. Natl. Acad. Sci. U.S.A. *95*, 11083 (1998).
7. Chang H.-C., Miyamoto H., Marwah P., Lardy H., Yeh S., Huang K.-E., Chang C.: Proc. Natl. Acad. Sci. U.S.A. *96*, 11173 (1999).
8. Miyamoto H., Marwah P., Marwah A., Lardy H., Chang C.: Proc. Natl. Acad. Sci. U.S.A. *100*, 4440 (2003).
9. Reich I. L., Lardy H., Wei Y., Marwah P., Kneer N., Powell D. R., Reich H. J.: Steroids *63*, 542 (1998).

SYNTHESIS OF 15β-SUBSTITUTED STEROIDS

RAISSA P. LITVINOVSKAYA, ALEXANDER V. BARANOVSKY, VLADIMIR A. KHRIPACH

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevicha, 5/2, 220141 Minsk, Belarus
E-mail: litvin@iboch.bas-net.by

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

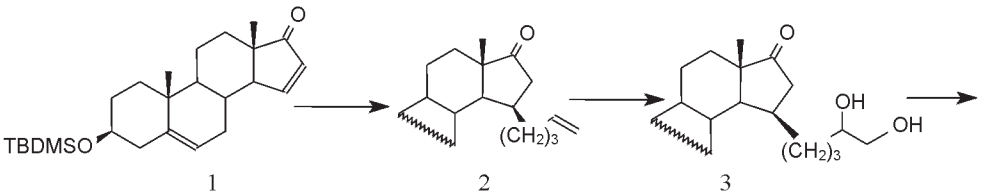
In this communication we discuss the application of androst-15-en 3-ethers such as 1 for the introduction of 15β-substituent containing oxygene. The title compounds were prepared according to the following scheme.

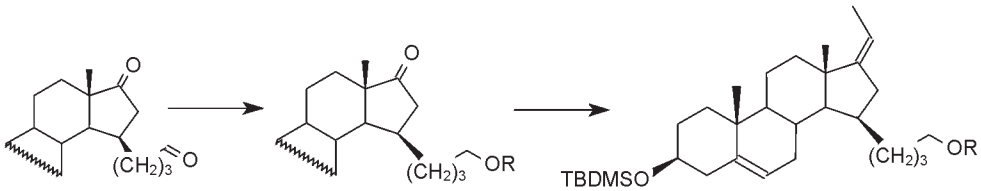
Details of preparation and identification procedures will be discussed.

It schould be noted that using of the obtained compounds 9–12 in ene reaction[1] opens a way to the corresponding 15β--substituted derivatives of pregnane and cholestane series.

REFERENCES

1. Mikami K., Shimizu M.: Chem. Rev. *92*, 1021 (1992).





	R
5	H
6	TBDMS
7	Piv
8	THP

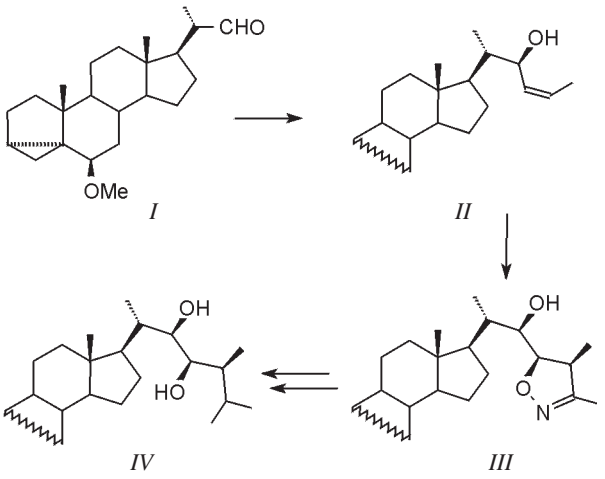
	R
9	TBDMS
10	Piv
11	H
12	THP

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

RAISSA P. LITVINOVSKAYA, MARINA A. AVERKOVA, NADEZHDA V. KOVAL, ALEXANDER V. BARANOVSKY, ALEXANDER S. LYAKHOV, VLADIMIR A. KHRIPACH

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevicha, 5/2, 220141 Minsk, Belarus
E-mail: litvin@iboch.bas-net.by

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



I *II* *IV* *III*

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

Taking into account that four diastereomeric products could be formed in this reaction the stereochemistry of the isoxazoline *III* have been determined by X-ray structure analysis.

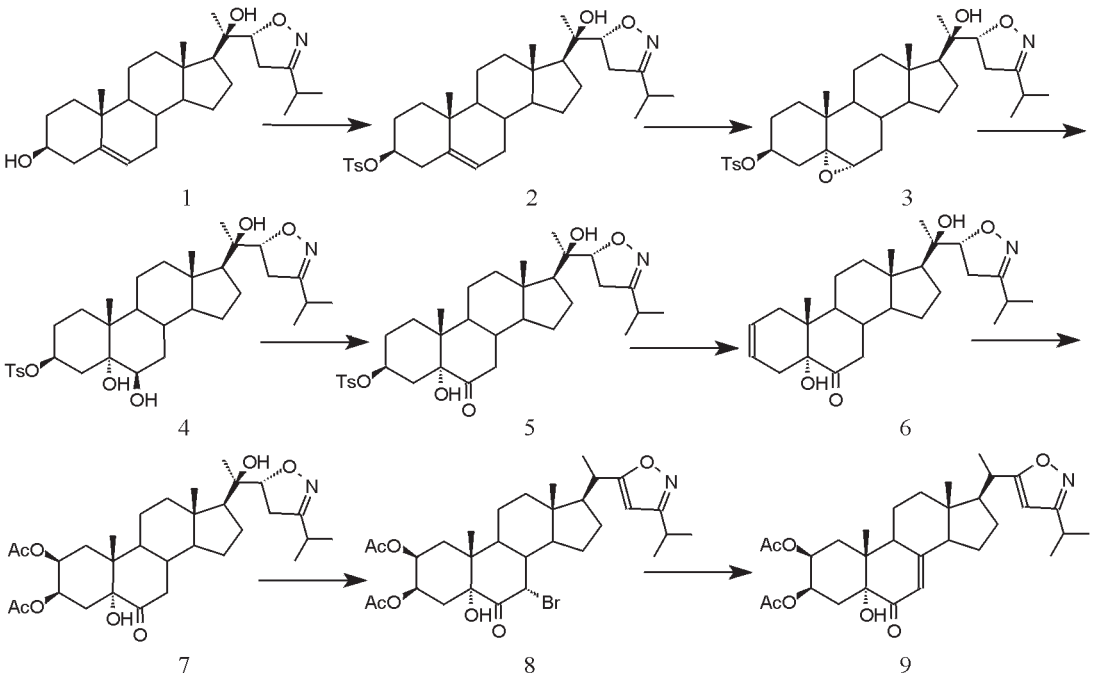
Some spectral and X-ray data, reaction mechanisms and chemical transformations of the isoxazolinylsteroid *III* into steroids with open side chain like *IV* will be discussed.

SYNTHESIS OF ISOXAZOLE ANALOGUES OF ECDYSTEROIDS

RAISSA P. LITVINOVSKAYA, SVETLANA V. DRACH, ELEHA N. MASALOVA, VLADIMIR A. KHRIPACH

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevicha, 5/2, 220141 Minsk, Belarus
E-mail: litvin@iboch.bas-net.by

Earlier we have shown that the application of isoxazolinylsteroids as key compounds allows effectively to form the



1 2 3 4 5 6 7 8 9

side chain of ecdysteroids, in particular ponasterone and pterosterone C (ref.[1]). In this work we have put the purpose to investigate an applicability of application 20-hydroxy-20-isoxazolinylsteroids such as 1 in reactions of formation of a cyclic moiety of ecdysteroid molecule.

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

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

REFERENCES

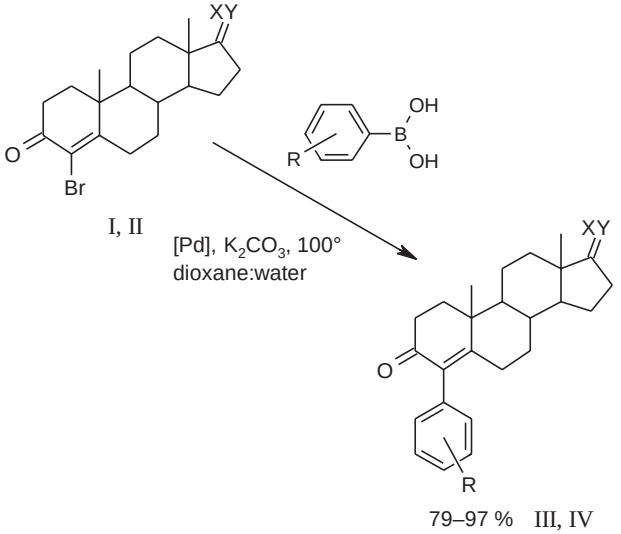
1. Khripach V. A., Litvinovskaya R. P., Baranovskii A. V.: Mendeleev Commun. *1992*, 117.

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

NIKOLAI V. LUKASHEV[a], GENNADY V. LATYSHEV[a], GEORGE A. SKRYABIN[b], IRINA P. BELETSKAYA[a]

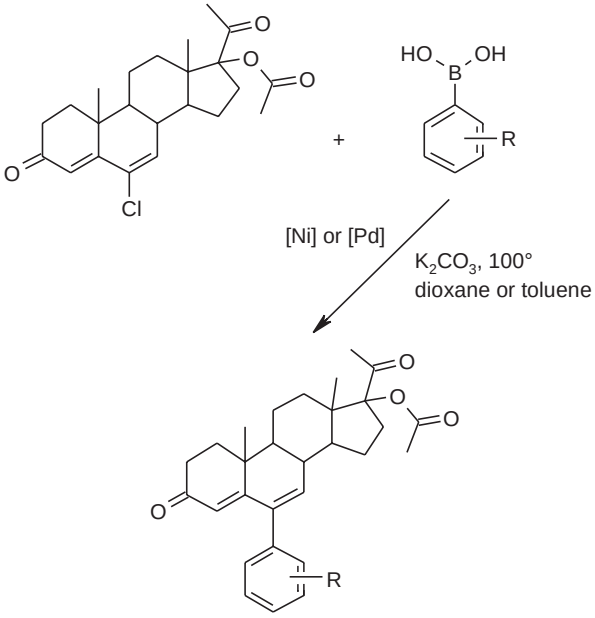
[a]The Department of Chemistry, Moscow State Lomonosov University, Vorobievy Gory, 119992, Moscow, Russia, [b]Ambiotech Inc., 300 E Lombard St. STE 1400 Baltimore MD 21202, USA
E-mail: lukashev@aha.ru, gskryabin@relline.ru

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



I, III: XY = O
II, IV: X = β-Ac, Y = β-OH

R = 4-MeO, 4-Me, 4-F, 4-COOH and others



R = 4-MeO, 4-Me, 4-F, yields 93–100 %
R = 3-Ac-, 4-COOH, yields 8–43 %

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

4-Carboxyphenyl substituent was found to be a convenient spacer group for binding above mentioned steroid molecules to protein carriers to prepare immunogens.

DETERMINATION OF LINEAR TERPENES ENANTIOMERS PRESENT AT LOW QUANTITITES IN NATURAL ULTI-COMPOUND MIXTURES

ANNA LUXOVÁ[a], ANNA-KARIN BORG--KARLSON[b], KLÁRA URBANOVÁ[a], IRENA VALTEROVÁ[a]

[a]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic, [b]Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden
E-mail: luxova@uochb.cas.cz

Chiral linear terpenic alcohols such as citronellol, 2,3-dihydrofarnesol and geranylcitronellol play an important role in interspecies chemical communication of bumblebees[1]. These compounds were also identified in scents of several flowers that are pollinated by bumblebee as well. It is important to know the ratio of enantiomers in chiral natural products. In many cases, interactions between receptor proteins in insect antenna with just one specific enantiomer of pheromone constituent were proved[2].

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

For separation of the linear terpene enantiomers from extracts of male LG and from scent of orchids, a two-dimensional gas chromatographic (2D-GC) technique[3] was used. This technique is crucial e.g. in case of determination the enantiomeric ratio of key monoterpenes in oviposition attractants of the *Cameraria ohridella* host plant *Aesculus hippocastanum*. This system represents an enantiospecific reaction between antennal receptor proteins of pest moth *C. ohridella* and kairomone of the horse chestnut.

Stationary phase in chiral column for separations of linear terpenic alcohols was 60 % of (heptakis(2,3-di-*O*-acetyl-6-*O*--TBDMS)-β-cyklodextrin) in PolysiloxanePS-86 (ref.[4]). A good separation of enantiomers of citronellol and 2,3-dihydrofarnesol was reached at the temperature around 110 °C. Higher pressure of carrier gas was used to reduce extensive retention time of 2,3-dihydrofarnesol.

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

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

This work was supported by the Grant Agency of the Czech Republic (grant No. 203/02/0158).

REFERENCES

1. (a) Valterová I., Svatoš A., Hovorka O.: Collect.Czech. Chem. Comunn. *61*, 1501 (1996); (b) Hovorka O., Urbanová K., Valterová I.: J. Chem. Ecol. *24*, 183 (1998).
2. Gessner M, Deger W, Mosandl A.: Z. Lebensm.–Unters. Forsch. *186*, 417 (1988).
3. Borg-Karlson A.-K., Lindström M., Norin T., Persson M., Valterová I.: Acta. Chem. Scand. *47*, 138 (1993).
4. Bratschat D., Kuntz C., Heil M., Schittrigkeit A., Schumacher K., Mang M., Mosandl A., Kaiser R.: Phytochem. Anal. *8*, 159 (1997).

STUDIES ON BRASSINOSTEROIDE HORMONE BINDING PROTEINS FROM PLANTS

TOMÁŠ MACEK[a], MAREK KAMLAR[a,b], RUDOLF JEŽEK[a], ANDREA PIŠVEJCOVÁ[b], CSABA KONCZ[c], LADISLAV KOHOUT[a]

[a]Department of Natural Products, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, [b]Department of Biochemistry and Microbiology, ICT Prague, Technická 3, 166 28 Prague 6, Czech Republic, [c]Max-Planck-Institut für Zuechtungsforschung, Carl-von-Linne-Weg 15, 50829 Koeln, Germany
E-mail: tom.macek@uochb.cas.cz

Brassinosteroids (BRs) are a group of plant steroids, of which the molecular and biochemical analysis of *Arabidopsis* mutants has furnished conclusive evidence that these compounds are plant growth hormones[1]. They are biologically active in the various bioassay systems designed for gibberellins, auxins and cytokinins, eliciting remarkable growth responses[2]. The molecular mechanism of BRs action is uncertain, although one might argue from structural considerations that they are likely to work by a mechanism similar to that of animal steroid hormones, which generally act *via* a soluble receptor-ligand complex that binds to nuclear sites to regulate the expression of specific genes. Despite many studies on plant steroids there is no report on successful isolation of a receptor. Recently published opinion is, that BRs act in plants after binding to a sterol binding protein (SBP), which complexes with receptor in the membrane, but also binding of BRst to the membrane receptor alone is not excluded[3].

In order to isolate the BRs and oxysterol-binding proteins or receptors we prepared brassinosteroid based bioaffinity ligands. Affinity chromatography carrier matrices obtained by oriented immobilisation of BRs ligands bound covalently by a proper spacer arm were compared for performance with plant extracts. The columns were used to isolate enough proteins from plant extracts for sequencing. Just now the final goal is cloning of receptors using methods of reverse genetics.The ligand must be bound by that part of the molecule which least participates in the biospecific binding. As far as it is not yet known exactly, which parts of the BRs molecule are actually necessary for the proper biological activity and which ones for specific binding, oriented immobilisation of different ligands to matrix was necessary. Among others newly synthesi-

sed brassinosteroid, (20*S*)-2α,3α-dihydroxy-7-oxa-B-homo--5α-pregnan-6-one-20-carboxylic acid, was used for immobilisation. This compound was obtained in eight steps by general synthesis of brassinosteroid skeleton[4] from bisnorcholanic acid. Various new BRs derivatives, tested also for other activities[5], were used to obtain chromatography carriers with BRs bound through the ring A, ring B or the side chain. Different carriers were tested to obtain acceptable yield of proteins in amounts sufficient for analysis of their primary structure.

The plant extracts were obtained by grinding frozen plant leaves or callus tissue, salts were removed by gel filtration and the extract applied to the bioaffinity matrix. The analysis of primary structures by sequencing of proteins obtained is underway. One of the proteins separated from *Nicotiana tabacum* callus extract was identified as osmotin-like protein precursor. The aminoacid sequence obtained showed a 100 % agreement with this pathogen-related (PR) protein, which appears in tobacco under stress conditions[6]. Our results thus reveal a connection between brassinosteroid and protein involved in stress response.

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

REFERENCES

1. Szekeres M., Nemeth K., Kon Kalman Z., Mathur J., Kauschmann A., Altmann T., Redei G. P., Nagy F., Schell J., Koncz C.: Cell *85*, 171 (1996).
2. Kohout L.: Collect. Czech. Chem. Commun. *59*, 457 (1994).
3. Mussig C., Altmann T.: Trends Endocrinol. Metab. *12*, 398 (2001).
4. Kohout L., Chodounská H., Macek T., Strnad M.: Collect. Czech. Chem. Commun. *65*, 1754 (2000).
5. Nešněrová P., Svatoš A., Kohout L., Macek T.: Plant Physiol. Biochem. *38*, S08 (2000).
6. Abad L., D'Urzo M. P., Liu D., Narasimhan M. L., Reuveni M., Zhu J. K., Niu X., Singh N. K., Hasegawa P. M., Bressan R. A.: Plant Sci. *118*, 11 (1996)

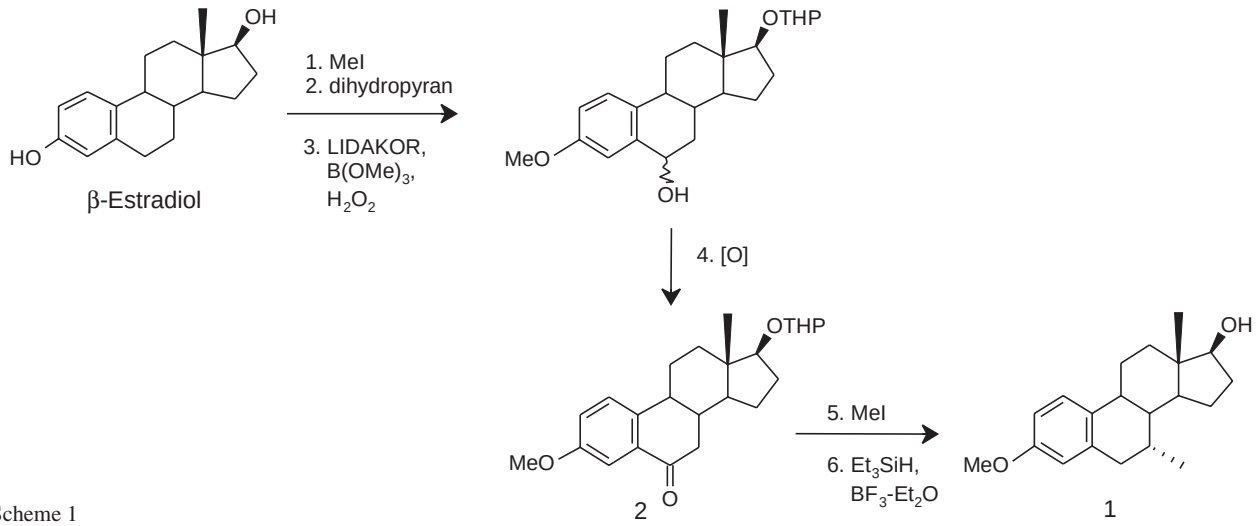
THE SYNTHESIS OF 17β-HYDROXY-3--METHOXY-7α-METHYL-1,3,5(10)-ESTRATRIENE FROM 17β-HYDROXY-4-ESTREN-3-ONE

JACEK MARTYNOW, ANDRZEJ KUTNER, HANNA FITAK

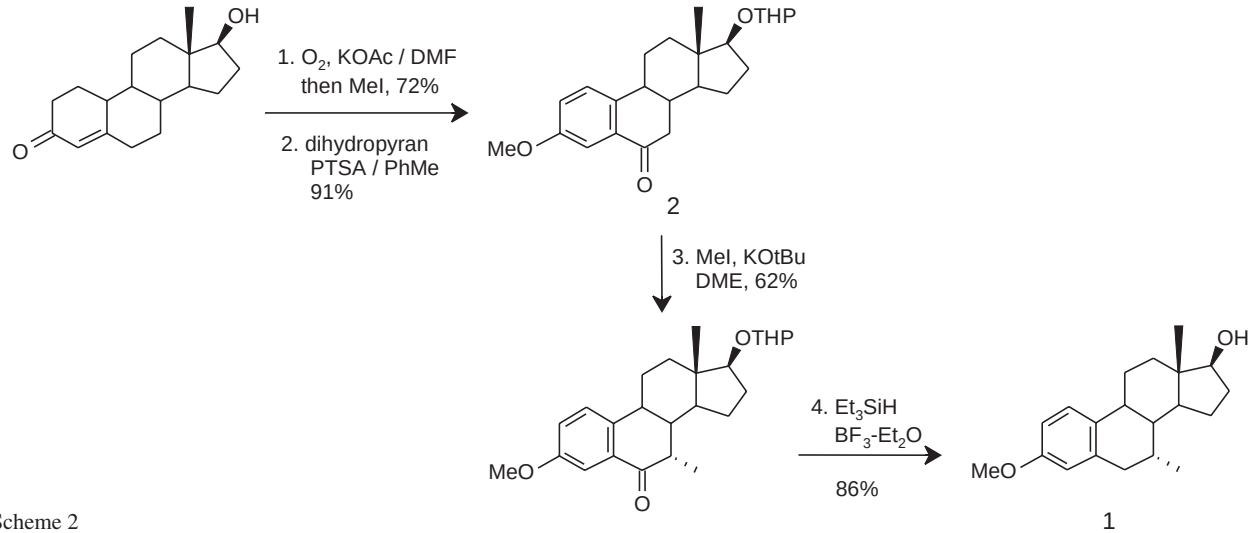
Pharmaceutical Research Institute, 8 Rydygiera Str., 01-793 Warsaw, Poland
E-mail: marsgm@poczta.onet.pl

17β-Hydroxy-3-methoxy-7α-methyl-1,3,5(10)-estratriene 1 is a key intermediate useful in the synthesis of a number of modified steroids, many of which display important biological activities[1]. Compound 1 is not commercially available in quantity – its preparation from more common steroids has been the subject of a few published synthetic protocols[2], which, however, are lengthy. The most straightforward synthesis of 1 disclosed to date appears to be the route based on the transformations of β-estradiol to 3-methoxy--17β-(tetrahydropyranyl)oxy-1,3,5(10)-estratrien-6-one 2, which subsequently is methylated at C(7), followed by a 17--*O*-deprotection and a C(6)-deoxygenation. This procedure affords 17β-hydroxy-3-methoxy-7α-methyl-1,3,5(10)-estratriene 1 in six steps from estradiol and in yields close to 40 % (ref.[3]).

However, we found that the synthesis of the 6-oxo derivative 2 according to *Scheme 1* is very troublesome at the C(6) hydroxylation stage, due to the formation of boron-derived side products which invariably create serious problems during the work-up. Thus, we felt that an improved synthesis of compound 2 from commercial steroids was necessary for a more practical route to 17β-hydroxy-3-methoxy-7α-methyl--1,3,5(10)-estratriene 1. In this context, we investigated a number of chromium(VI)-based oxidation methods[4], starting from estradiol or its OH-protected analogues. All these attempts in our hands were essentially futile – low yields and poor selectivities were observed, perhaps with the exception of the PDC/Pyr conditions[4d], which required long reaction times and



Scheme 1



Scheme 2

gave yields below 60 %. Similarly inefficacious proved the approach based on oxidation of 17β-hydroxy-4-estren-3-one by molecular oxygen in DMSO, in the presence of a base[5]. In light of these results, it was very gratifying for us to find that 17β-hydroxy-4-estren-3-one under the action of molecular oxygen in hot DMF, in the presence of potassium acetate[6] was quite cleanly transformed to 3,17β-dihydroxy-1,3,5(10)-estratrien-6-one. The latter compound, in the same reaction pot, was subsequently selectively methylated at the phenolic OH, followed by a separate step of tetrahydropyranylation at the remaining hydroxyl. In our hands, this procedure – carried out on a 20 g scale – gave the desired compound 2 in a 65 % yield from 17β-hydroxy-4-estren-3-one (Scheme 2).

We completed the synthesis of 17β-hydroxy-3-methoxy--7α-methyl-1,3,5(10)-estratriene 1 on the way of α-methylation of the 6-oxo derivative 2 (MeI, KOtBu, DME: 62 % yield after chromatography), followed by C(6)-deoxygenation and 17-*O*-deprotection (Et_3SiH, $BF_3.Et_2O$: 86 %). This gave the key compound 1 (crystalline) in 35 % total yield from 17β--hydroxy-4-estren-3-one, in only four synthetic steps (Scheme 2). Equivalent conditions[7] (Et_3SiH-TFA) for the deoxygenation reaction of similar C(6)-ketones have also been reported. The direct synthesis of compound 1 from 17β-hydroxy-4--estren-3-one presented here is conceptually simple and, quite possibly, it is the most practical of all the disclosed approaches to 17β-hydroxy-3-methoxy-7α-methyl-1,3,5(10)-estratriene.

REFERENCES

1. Lednicer D., Mitscher L. A.: *The Organic Chemistry of Drug Synthesis*, Vol. 2, Chap. 6. Wiley, New York1980.
2. (a) Wieland P., Anner G.: Helv. Chim. Acta *50*, 289 (1967); (b) Anner G., Wieland P.: FR 1418540 19651119.
3. (a) Skaddan M. B., Wüst F. R., Katzenellenbogen J. A.: J. Org. Chem. *64*, 8108 (1999); (b) Tedesco R., Katzenellenbogen J. A., Napolitano E.: Tetrahedron Lett. *38*, 7997 (1997); (c) Tedesco R., Fiaschi R., Napolitano E.: Synthesis *1995*, 1493; (d) Sala A., Lenna R.: IT 2000MI0918 A1 20011022.
4. (a) PCC/Celite: Mons S., Lebeau L., Mioskowski C.: Synth. Commun. *28*, 213 (1998); (b) CrO_3/AcOH: Dean P. D. G., Exley D., Johnson M. W.: Steroids *30*, 593 (1971); (c) CrO_3-DMP: Garza G. A., Rao P. N.: Steroids *42*, 3188 (1983); (d) PDC/Pyr: Bastiaansen P. M. F. M., Kohout L., Posthumus M. A., Wijnberg J. B. P. A., de Groot A.: J. Org. Chem. *61*, 859 (1996).
5. Hofmeister H., Laurent H., Wiechert R.: Chem. Ber. *106*, 723 (1973).
6. Plate R., van Wuijtswinkel R. C. A. L., Jans C. G. J. M., Groen M. B.: Steroids *66*, 117 (2001).
7. Rao P. N., Cessac J. W., Kim H. K.: Steroids *59*, 621 (1994).

In vitro ASSESSMENT OF THE NEUROACTIVE POTENTIAL OF PREGNANE DERIVATIVES ON $GABA_A$ RECEPTOR USING PRIMARY CULTURES OF CORTICAL NEURONS

LIBOR MATYÁŠ[b,c], ZOILA BABOT[a], ALEXANDER KASAL[b], CRISTINA SUÑOL[a*]

[a]Department of Neurochemistry, Institut d'Investigacions Biomèdiques de Barcelona, Consejo Superior de Investigaciones Científicas (CSIC, IDIBAPS), Rosselló 161, Barcelona, Spain, [b]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, [c]Institute of Chemical Technology, 166 28 Prague 6, Czech Republic
E-mail: csenqi@iibb.csic.es

The steroids synthesized by the brain and nervous system, named *neurosteroids*, have a wide variety of diverse functions. In general, neurosteroids mediate their actions, not through classic steroid hormone nuclear receptors, but through other mechanisms such as through ion gated neurotransmitter receptors. In the adult, neurosteroid stimulation of neurotransmitter receptors results in behavioral effects, such as decreased an-

xiety, sedation, and decreases in seizure activity, these effects being associated with stimulation of neuronal $GABA_A$ receptors (for review, see[1,2]). Thus, one of the primary neurosteroid target receptors considered is the γ-aminobutyric acid type A ($GABA_A$) receptor complex.

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

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

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

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

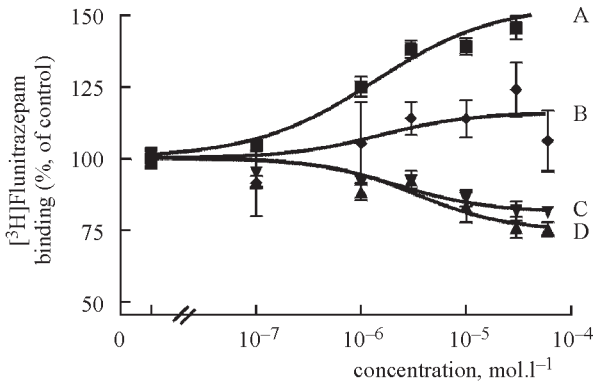


Fig. 1. A. 3α-OH-5α-pregnan-one
B: 3α-fluoro-2β-OH-5α-pregnan-one
C and D: CMO derivatives of 3α-fluoro-5α-pregnan-one

decreased [^{3}H]flunitrazepam binding by 20–25 %. The accompanying Fig. 1 shows the effects of these compounds.

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

Supported by FIS grant 01/1318 and CSIC – Academy of Sciences agreement project 2003CZ0005. Z. Babot and L. Matyas are recipients of a fellowship from the Spanish Ministries of Education, Culture and Sports and Foreign Offices, respectively.

REFERENCES

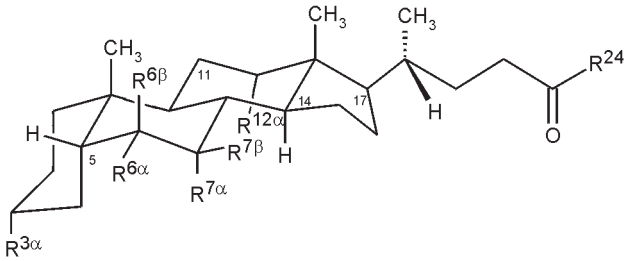
1. Plassat-Schiess E., Baulieu E. E.: Brain Res. Rev. *37*, 133 (2001).
2. van Broekhoven F., Verkes R.: Psychopharmacology *165*, 97 (2003).
3. Rupprecht R., Holsboer F.: Trends Neurosci. *22*, 410 (1999).
4. Suñol C., Pomés A., Rodríguez-Farré E., in: *European Medicines Research. Perspectives in Pharmacotoxicology and Pharmacovigilance* (Fracchia G. N., ed.), pp. 223–234. IOS Press, 1994.
5. Pomés A., Rodríguez-Farré E., Suñol C.: Dev. Brain Res. *73*, 85 (1993).
6. Pomés A., Rodríguez-Farré E., Suñol C.: J. Pharmacol. Exp. Ther. *271*, 1616 (1994).
7. Vale C., Pomés A., Rodríguez-Farré E., Suñol C.: Eur. J. Pharmacol. *319*, 343 (1997).
8. Vale C., Vilaró E., Rodríguez-Farré E., Suñol C.: J. Neurosci. Res. *57*, 95 (1999).

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

RUDOLF MEGGES[a,b*], RUDOLF SCHÖN[a], JÜRGEN WEILAND[a], HEINRICH REPKE[b], KURT R. H. REPKE[a]

[a]Max Delbrück Center for Molecular Medicine, D-13092 Berlin-Buch, Germany, [b]German-American Institute for Applied Biomedical Research GmbH (GAIFAR), D-14 473 Potsdam, Germany
E-mail: mmegges4@compuserve.de

The Na^+/K^+-ATPase – the molecular point of attack of cardioactive steroids – has been shown by us to be suited for the analysis of steroids of different types as to their respective proper-ties[1]. Cholanic acids (bile acids) have the favourable 5β configuration and a 17β substituent with the same number of carbon atoms as the highly cardioactive bufadienolides. Therefore, they are potential and available starting compounds for the synthesis of cardioactive drugs despite of the less favourable 3α-OH and 14α-H (= C/D-*trans* connection). We have investigated 9 cholanic acid methyl esters (CA) carrying up to 3 OH groups and one amide with respect to their inhibition of human kidney Na^+/K^+-ATPase (Table I).



$R^{3\alpha}$ = -OH (1,3–10); =-OAc; Δ^{11} = (2)
R^{24} = -OCH_3 (1,2,4–10); -$NHCH_2CO_2CH_3$ (3)

1: 3α-hydroxy-5β-cholan-24-oic acid methyl ester (*lithocholic acid methyl ester*)
2: 3α-acetoxy-5β-chol-11-en-24-oic acid methyl ester (*lithocholenic acid methyl ester 3-acetate*)
3: 3α-hydroxy-5β-cholan-24-oic acid-*N*-(methoxycarbonyl-methyl) amide
4: 3α,6α-dihydroxy-5β-cholan-24-oic acid methyl ester (*hyodeoxycholic acid methyl ester*)
5: 3α,6β-dihydroxy-5β-cholan-24-oic acid methyl ester (*murocholic acid methyl ester*)
6: 3α,7α-dihydroxy-5β-cholan-24-oic acid methyl ester (*chenodeoxycholic acid methyl ester*)
7: 3α,7β-dihydroxy-5β-cholan-24-oic acid methyl ester (*ursodeoxycholic acid methyl ester*)
8: 3α,12α-dihydroxy-5β-cholan-24-oic acid methyl ester (*deoxycholic acid methyl ester*)
9: 3α,6α,7α-trihydroxy-5β-cholan-24-oic acid methyl ester (*hyocholic acid methyl ester*)
10: 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid methyl ester (*cholic acid methyl ester*)

Table I
Equilibrium and kinetic constants[1,4–6] of substances in the Na^+/K^+-ATPase (human kidney) inhibition test. k_{on} or k_{off} = velocity constant for formation or decay of the effector-receptor complex, respectively. $K'_D = k_{off}/k_{on}$ = inhibition constant at 37 °C and pH 7.4. sl = solubility, r.a.= relative activity (1 = 100).

	k_{on} [$\mu M^{-1}.min^{-1}$]	k_{off} [min^{-1}]	K'_D [μM]	sl [μM]
1	0.0044	0.18	41	15
2[a]			>>20	20
3	0.011	0.52	48	80
4	0.0080	0.13	15.7	50
5	0.0047	0.19	40	50
6	0.0080	0.13	15.7	50
7	0.0028	0.20	69	50
8	0.014	0.22	15.7	80
9	0.0032	0.26	80	100
10	0.0024	0.33	138	>300

[a] No inhibition up to solubility limit

Compound 1 has only one equatorial 3-OH group which is common to all investigated CA except 2. Introduction of a second OH group increases [4: 6α- (eq), 6: 7α- (ax), or 8: 12α-OH (ax)] or decreases [7: 7β-OH (eq)] the activity compared to 1, whereas 6β-OH (ax) in 5 has no influence. Thus, improvement of the activity is caused by one OH-group at the α-face of the steroid backbone. A third additional OH group [9: 6α-(eq), 7α-OH (ax)], 10: [7α- (ax), 12α-OH (ax)] did not further increase but decreases the activity. The 3α-*O*-acetyl--Δ^{11} derivative of 1 (2) shows a strong decrease of activity. Exchange of -$COOCH_3$ in 1 against -$CONHCH_2$-$COOCH_3$ (3) shows nearly no influence on the activity. This is remarkable, as O → N exchange in the lactone rings of cardenolides[2] or bufadienolides[3] shows a strongly decreased activity of the lactames compared to the oxygen analogues. Compared to the cardioactive compounds bufalin[1] or digitoxigenin[1] or canrenone[4], the activity of 1 is about 4 or 3 orders of magnitude lower or one order higher, respectively. These differences reflect the differences in the velocity of the ATPase effector receptor complex formation (k_{on}) whereas the velocities of complex decay (k_{off}) are similar.

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

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

REFERENCES

1. Repke K. R. H., Megges R., Weiland J., Schön R.: Angew. Chem., Int. Ed. Engl. *34*, l294 (1995); Angew. Chem. *107*, 308 (1995).
2. Megges R., Weiland J., Schön R., Repke H., Repke K. R. H.: Collect. Czech. Chem. Commun. *67*, 336 (2002).
3. Megges R., Weiland J., Portius H.-J., Glusa E., Messerschmid A., Repke H., Repke K. R. H.: *23rd IUPAC-2002*

Int.Symposium on the Chemistry of Natural Products, Florence/Italy, 28 July–2 August, 2002, Book of Abstracts, p. 274.
4. Repke K. R. H., Schön R., Weiland J., Megges R., Nitz M.: German Patent (DE-Offenlegungsschrift) 43 21 937 (1995).
5. Repke K. R. H., Sweadner K. J., Weiland J., Megges R., Schön R.: Prog. Drug. Res. *47*, 9 (1996).
6. Repke K. R. H., Schönfeld W., Weiland J., Megges R., Hache A., in: *The Design of Enzyme Inhibitors as Drugs* (Sandler M., Smith H. J., ed.), pp. 435–502. Oxford University Press, Oxford 1989.

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

MICHAŁ MICHALAK, KAROL MICHALAK, JERZY WICHA

Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

The dicyclopenta[*a*,*d*]cyclooctene ring system is the structural core of numerous diterpenes (such as the fusicoccin[1]) and sesterterpenes (such as the ophiobolins and ceroplastin[2]). The wide range of biological activities exhibited by these compounds[1,2] and their specific structural features have stimulated interest in developing synthetic approaches to their core ring system. In this communication we present a new approach to synthesis of 5–8 ring carbon framework using three consecutive reactions: Mukaiyama-Micheal conjugate addition, palladium-catalyzed allylation (Tsuji alkylation) and ring closing metathesis (RCM). The reactivity differences in 8-membered ring closure due to stereochemical factors will be discussed.

REFERENCES

1. Leung P. C., Taylor W. A.,Wang J. H., Tripton C. L.: J. Biol. Chem. *259*, 2742 (1984).
2. Tsipouras A. A., Tkacz J. S., Frazier E. G, Rohrer S. P., Birzin E., Rosegay A., Zink D. L., Goetz M. A., Singh S. B., Schaeffer J. M.: Bioorg. Med. Chem. *4*, 53 (1996).

BIOTRANSFORMATION OF (+)- AND (–)-CARVONES BY CELERIAC AND CARROT ENZYMATIC SYSTEM

AGNIESZKA MIRONOWICZ, WANDA K. MĄCZKA

Department of Chemistry, Agricultural University, ul. Norwida 25, 50-375 Wrocław, Poland
E-mail: amiron@ozi.ar.wroc.pl

S-(+)-carvone, having the scent of caravay, and *R*-(–)-carvone, which smells of green pepper, are commonly present in many plants (*Mentha spicata*, *M. viridis*, *Cyperus rotundus* root). These compounds are used as fragrants and flavourings, and as drugs – in therapeutics based on natural plant extracts (*Carum carvi* L., *Anethum graveolens* L.). *R*-(–)-carvone is considered a very promising inhibitor of farnesyltransferase (FTase; IC_{50}= 1,5 mM) – an enzyme that attends in post-translational prenylation of Ras proteins, which are responsible for cells proliferation, to function properly. Carvone and dihydrocarveol are active against Gram– and Gram+ bacteria.

Carvone and its derivatives are also used as starting materials for the synthesis of many drugs, e.g. antimalarial and anticancer agents or calcium metabolism regulators.

Our interests have been focused on obtaining derivatives of 4*R*-(–)- and 4*S*-(+)-carvone with precisely established absolute configuration at C-1 and C-2 stereocentres. These have potential applications as valuable chiral synthons for asymmetric synthesis. The most common pathway for carvone biotransformations described in literature (except for hydroxylation reactions) comprises a two-step process leading to carveols. The first step is the reduction of the C=C double bond in the cyclohexene ring, followed by the reduction of the carbonyl group[1].

In our studies we used enzymatic systems of celeriac (*Apium graveolens* L., var. *rapaceum*) and carrot (*Daucus carota* L.) in order to obtain 4*R*-(–)- and 4*S*-(+)-carvone derivatives in high optical purity. The biocatalysts we used transformed 4*R*-(–)-carvone to dihydrocarveol also in two steps, but in the opposite order. The carbonyl group was reduced first to give an allyl alcohol (2*S*-carveol), and then the reduction of the double bond in the ring occurred, which led to 1*R*-dihydrocarveol.

Transformation of 4*S*-(+)-carvone proceeded in a different manner: the carbonyl group remained intact with 1*R*-dihydrocarvone as the only product.

REFERENCES

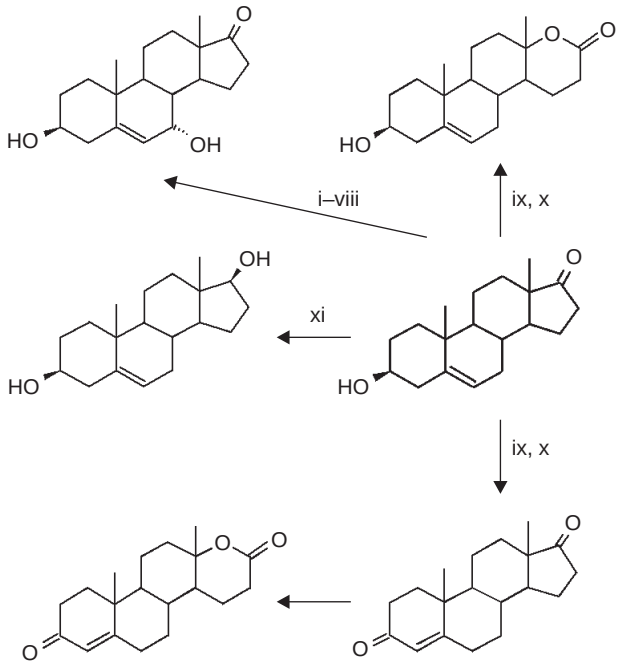
1. Speicher A., Roeser H., Heisel R.: J. Mol. Catal. B: Enzym. *22*, 71 (2003).

FUNGAL CONVERSION OF DEHYDROEPIANDROSTERONE

ANNA SZPINETER*, TERESA KOŁEK, ALINA ŚWIZDOR, AGNIESZKA MIRONOWICZ

Department of Chemistry, Agricultural University of Wrocław, ul. Norwida 25, 50-375 Wrocław, Poland
E-mail: szpinet@star.ar.wroc.pl

Dehydroepiandrosterone (DHEA) is a secretory product of adrenal, gonads and the central nervous system. Part of general pule of DHEA is derived from its circulating sulfate ester (DHEAS)[1]. Serum DHEA concentrations decrease with age and this fact leads to speculations about a possible positive role of DHEA hormone in aging. In humans DHEA is a precursor of sex steroid hormones such as androgens and estrogens. DHEA can influence processes of cognition and memory due to its neuroactive effect. Administration of this hormone to elderly people improves a frame of mind, mood and sexuality[2].This compound can play an important role in enhancing the immune system response and improving cell proliferation control[3].



Scheme 1. (i) *Absidia coerulea*; (ii) *Fusarium culmorum*; (iii) *Fusarium oxysporum*; (iv) *Nigrospora oryzae*; (v) *Aspergillus ochraceus*; (vi) *Mucor hiemalis*; (vii) *Mucor circinelloides*; (viii) *Penicillium frequentans*; (ix) *Penicillium camembertii*; (x) *Penicillium lilacium*; (xi) *Botrytis cinerea*

Human and murine species are able to hydroxylate DHEA at 7α-position and the presence of product of this hydroxylation (7α-hydroxy-DHEA) was observed in liver, brain and other tissues. The 7α-hydroxylated derivative has stronger biological activity than DHEA and shows more effective activation of immune processes in mouse. It enhances the resistance against lethal infections and counteracts the glucocorticosteroid immune supression in peripheral tissues[4].

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

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

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

REFERENCES

1. Burger H. G.: Fertil. Steril. *77*, Suppl. 4, 3 (2002).
2. Allolio B., Arlt W.: Trends Endocrinol. Metab. *13*, 228 (2002).
3. Arlt W., Justl H. G., Callies F., Reincke M.: J. Endocrinol. Metab. *83*, 1928 (1998).
4. Morfin R., Lafaye P., Cotillon A. C., Nato F., Chmielewski V., Pompon D.: Ann. N.Y. Acad. Sci. *917*, 971 (2000).
5. Roberts S. M., Wan P. W. H.: J. Mol. Catal., B: Enzymatic *4*, 111 (1998).

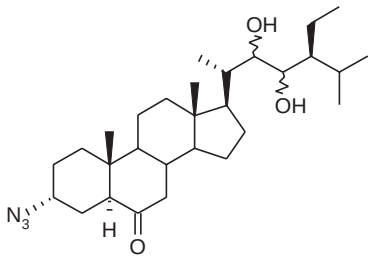
NEW BRASSINOSTEROID ANALOGS HAVING NITROGENATED FUNCTIONALITIES AT C3 TO PROVIDE MORE INFORMATION ABOUT THE BRASSINOSTEROID-RECEPTOR INTERACTION

MERITXELL MOLIST, MARC AMORÓS, CRISTINA DAVI, XEVI HERNÀNDEZ, LAURA TOBELLA, CARME BROSA*

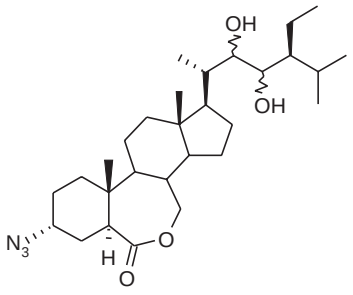
Institut Químic de Sarrià, C.E.T.S., Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain
E-mail: brosa@iqs.url.es

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

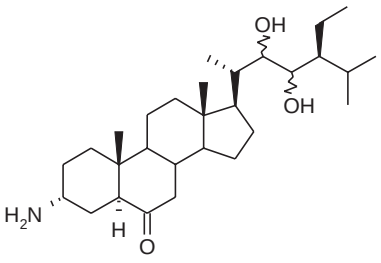
Considering that hydrogen bonding interaction can take place in the brassinosteroid-receptor complex[2], interesting points of view to be determined are: (*1*) whether the OH groups present in an active brassinosteroid act as acceptors or as donors in such hydrogen bonding, (*2*) the contribution of each OH group presents in the brassinosteroid to develop biological activity.



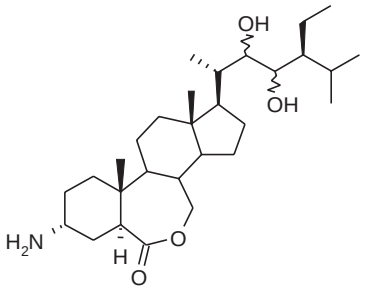
I: 22*R*,23*R*
II:22*S*,23*S*



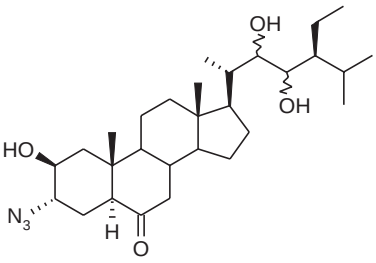
III: 22*R*,23*R*
IV:22*S*,23*S*



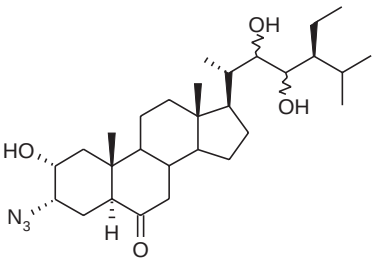
V: 22*R*,23*R*
VI:22*S*,23*S*



VII: 22*R*,23*R*
VIII:22*S*,23*S*



IX: 22*R*,23*R*
X:22*S*,23*S*



XI: 22*R*,23*R*
XII:22*S*,23*S*

In this sense and focused on the A ring, the substitution of the OH function at C3 by another functional group, amine or azide and the activity evaluation of such compounds should give us more information about the type of interaction that could take place upon binding in such positions. Moreover, analogs with the presence or not of an adjacent OH at C2 would be useful to extend the number of compounds with modifications on the A ring and useful to clarify the contribution of OH-C2 and OH-C3 in the activity[3].

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

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

REFERENCES

1. Khripach V. A., Zhabinskii V. N., de Groot A. E.: *Brassinosteroid: A New Class of Plant Hormones*. Academic Press, California 1999.
2. Brosa C.: *Structure-activity Relationship in Brassinosteroids: Steroidal Plant Hormones* (Sakurai A., Yokota T., Clouse S., ed.) Chap. 9. Springer-Verlag, Tokyo 1999.
3. See poster Marc Amorós et al.

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

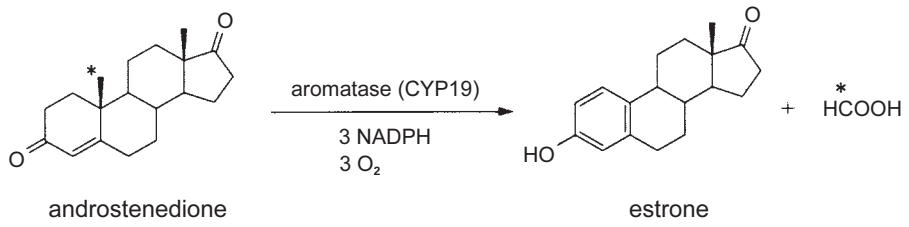
VINCENT C. O. NJAR, ANGELA M. H. BRODIE

Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, 685 West Baltimore Street, Baltimore, MD 10201-1559, USA

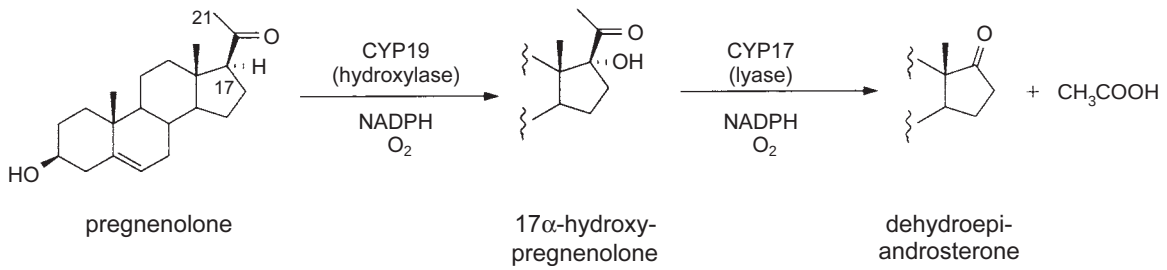
The cytochromes P450 constitutes a super family of heme--thiolate enzymes, present in all species[1]. The P450 enzymes involved in steroid hormone biosynthesis represent an important target for drug discovery and development for the treatment of hormone-dependent cancers (reviewed by Van Wauwe and Janssen[2]). The most extensively developed are the inhibitors of the enzyme cytochrome P450 aromatase (CYP19), responsible for the conversion of androgens into estrogens and a target in the treatment of breast cancer. The sequence of reactions catalyzed by CYP19 involves three sequential enzymatic hydroxylations (Scheme 1). The first two take place on the C-19 methyl group, whereas the final hydroxylation step is still unclear.

Because ~80 % of patients with prostate cancer have androgen-dependent diseases, that respond to hormonal ablation, the inhibition of androgen synthesis is also an important target for the treatment of prostate cancer. The last step in the biosynthesis of androgens involves the two-step conversion of pregnenolone and progesterone, *via* their corresponding 17α--hydroxy derivatives, to dehydroepiandrostenedione and androstenedione respectively. Both reactions are catalyzed by the same enzyme; cytochrome P450 17α-hydroxylase/17,20--lyase (CYP17)[3] (Scheme 2).

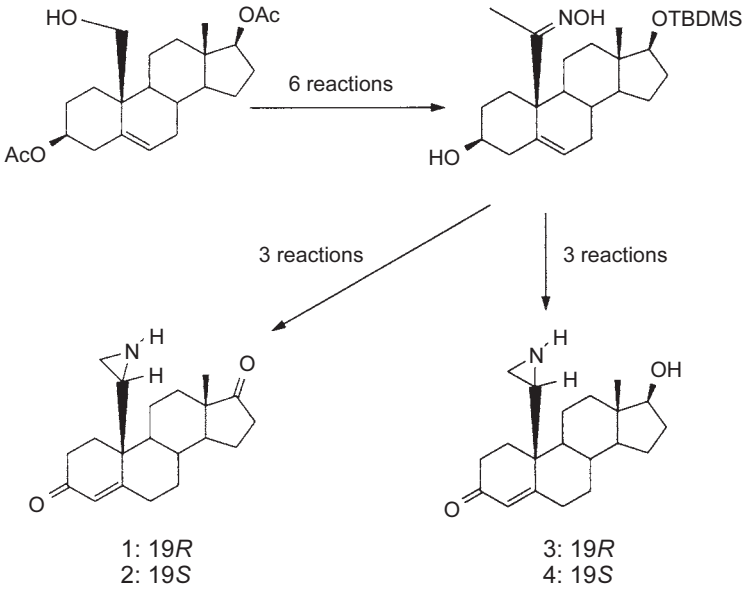
Extensive worldwide efforts have resulted in the identification of structurally diverse series of inhibitors of CYP19 and



Scheme 1. Action of aromatase (CYP19)



Scheme 2. Action of 17α-hydroxylase-17,20-lyase (CYP17)

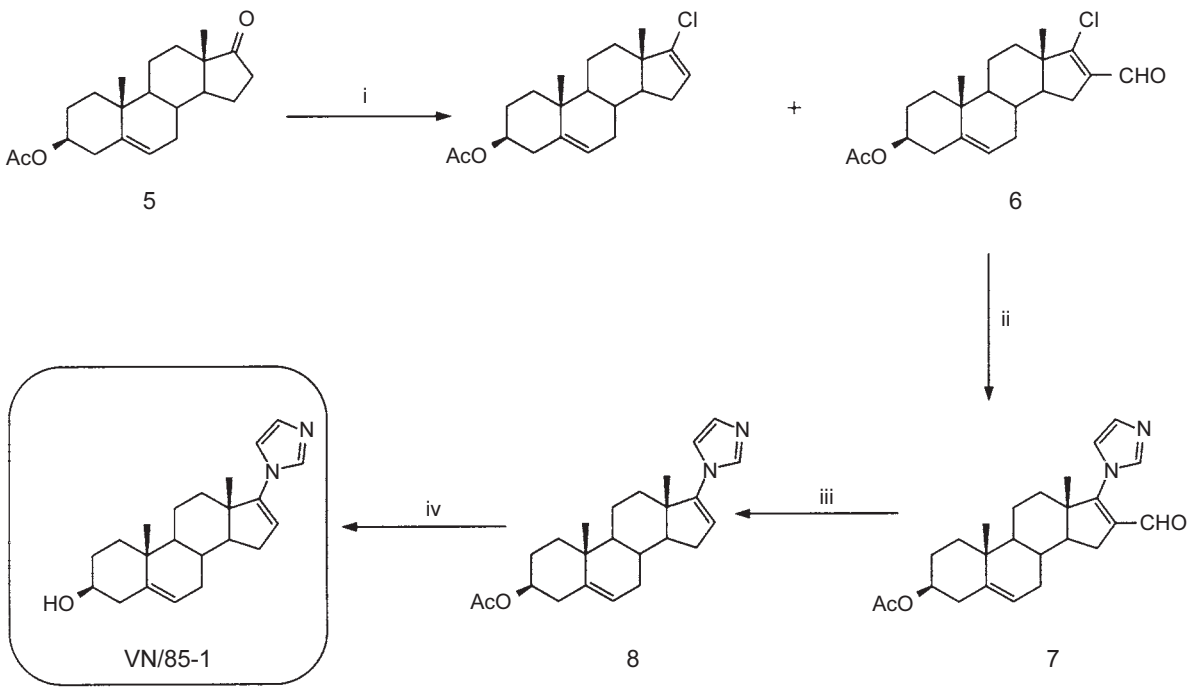


Scheme 3. Synthesis of 10β-aziridinyl steroids

of CYP17. Indeed, four CYP19 inhibitors, namely, 4-hydroxyandrostenedione (formestane), anastrazole, letrozole and exemestane are used clinically for the treatment of breast cancer (reviewed by Njar and Brodie[4]). A number of CYP17 inhibitors are currently in development as potential agents for the treatment of prostate cancer[5]. The present contribution will focus on our efforts in the rationale design, synthesis and evaluation of inhibitors of CYP19 and of CYP17. Specifically, we describe studies of steroidal inhibitors, involving functionalization (*N*-heterocycle) at either the C-19 (for CYP19 inhibitors) or the C-17 (for CYP17 inhibitors) positions in such a way as to mimic the natural substrates of the respective enzymes. The overall strategy is aimed at producing substrate-like compounds which are likely to not only interact with the steroid binding site of the enzyme, thus introducing high specificity, but also to provide a sixth ligand to the enzyme's heme iron resulting in tight binding.

Aromatase(CYP19) Inhibitors: The novel (19*R*)- and (19*S*)--10β-aziridinylestr-4-ene-3,17-diones 1 and 2 and the corresponding (19*R*)- and (19*S*)-10β-aziridinyl-17β-hydroxyestr-4--en-3-ones 3 and 4 (Scheme 3) have been prepared from the 19-oximino-19-methyl intermediate[6]. The key reaction was the conversion of the 19-oxime into the diastereomeric 10β--aziridines by lithium aluminium hydride (LAH). The synthetic route used is briefly summarized in Scheme 3. Rigorous establishment of the C-19 configuration in the aziridinyl steroids was secured by the X-ray crystallographic analysis of compound 3. The compounds were shown to be powerful and stereoselective inhibitors of human placental microsomal aromatase. We also showed that the nitrogen atom of the most potent aziridine coordinates to the enzyme's heme iron. These compounds are amongst the most potent inhibitors of this enzyme to date.

CYP17 Inhibitors: In our search for potent and selective inhibitors of CYP17, a variety of novel Δ^{16}-17-azolyl steroids, that is, pyrazoles, imidazoles, triazoles and tetrazoles have been prepared by a new synthetic route[7–9]. This involved the nucleophilic vinylic "addition-elimination" substitution reaction of 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene and azolyl nucleophiles. These series of azolyl steroids are unlike the heretofore known 17-heteroaryl steroids, as in this case, the azole moiety is attached to the steroid nucleus at C-17 *via* nitrogen of the azole. Most of the compounds are potent inhibitors of both the human and rat CYP17. The most potent compound, 3β-hydroxy-17-(1*H*-imidazole-1-yl)androsta-5,16--diene (VN/85-1), with a K_i value of 1.2 nM is 32 times more potent than ketoconazole (K_i = 38 nM). The synthetic route used to prepare VN/85-1 is briefly summarized in Scheme 4.



Scheme 4. (i) $POCl_3$-DMF, $CHCl_3$, Ar, reflux; (ii) imidazole, K_2CO_3, DMF, Ar, 80 °C; (iii) 10% Pd on activated charcoal, PhCN, reflux; (iv) 10% methanolic KOH, Ar, rt.

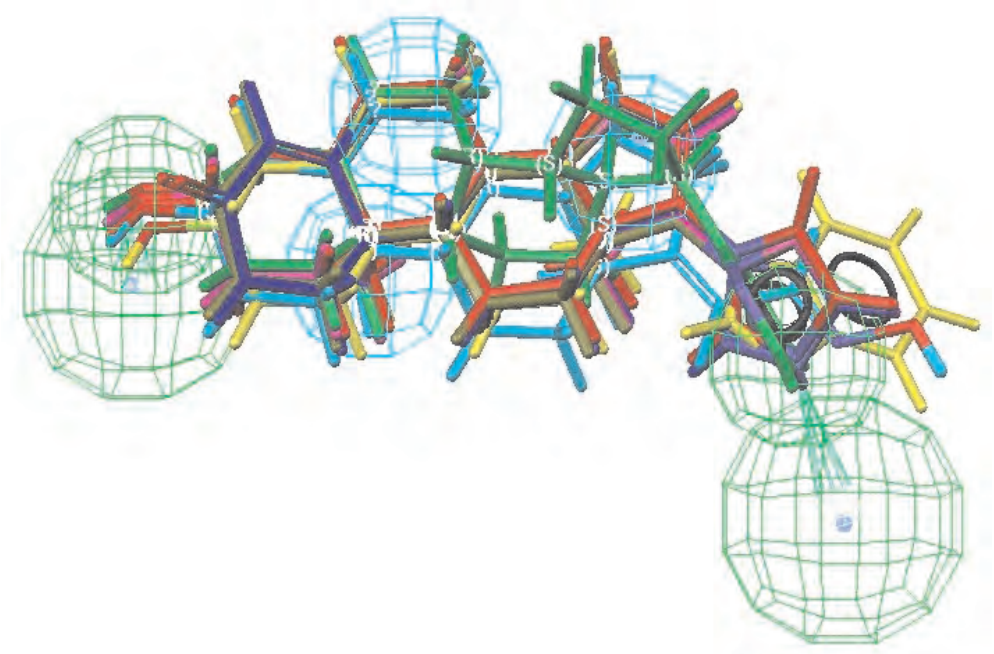


Fig. 1. Alignment of common-feature pharmacophore model with training set of CYP17 inhibitors

Spectroscopic studies with a modified form of human CYP17 indicate that the inhibition process involves binding of steroidal azole nitrogen to the heme iron of the enzyme. In cultures of human prostate cancer cell line (LNCaP), VN/85-1 effectively blocked the growth-stimulating effects of testosterone (T) and dihydrotestosterone (DHT), and was shown to manifest anti-androgenic activity[10]. In Sprague Dawley male rats, VN/85-1 suppressed T and DHT to basal levels after two 2 weeks of daily dosing at 50 $mg.kg^{-1}.day^{-1}$ (ref.[11]). Furthermore, remarkable antitumor activity was also observed against human prostate cancer (LNCaP) xenografted in combined immunodeficient (SCID) mice[10,12]. Our most potent inhibitors are currently in development.

In the absence of detailed structural information on the

CYP17 binding site, we have employed a ligand-based computational approach, by analyzing a variety of know CYP17 inhibitors, to identify ligand requirements for inhibiting this enzyme. Alignment of common-feature pharmacophore model with training set of CYP17 inhibitors is presented in Fig. 1. The study has provided the first insight into hypothetical binding requirements for steroidal and non-steroidal inhibitors of CYP17 enzyme[13]. The model may be useful in identification of new and potent CYP17 inhibitors.

REFERENCES

1. Nelson D. R., Koymans L., Kamataki T., et al.: Pharmacogenetics *6*, 1 (1996).
2. Van Wauwe J. P., Janssen P. A. J.: J. Med. Chem. *32*, 2231 (1989).
3. Hall P. F.: J. Steroid Biochem. Mol. Biol. *20*, 527 (1981).
4. Njar V. C. O., Brodie A. M. H.: Drugs *58*, 233 (1999).
5. Njar V. C. O., Brodie A. M. H.: Curr. Pharm. Design *5*, 163 (1999)
6. Njar V. C. O., Safi E., Silverton J. V., Robinson C. H.: J. Chem. Soc., Perkin Trans. 1 *1993*, 1161.
7. Njar V. C. O., Klus G. T., Brodie A. M. H.: Bioorg. Med. Chem Lett. *6*, 2777 (1996).
8. Njar V. C. O., Kato K., Nnane I, P., Grigoryev D. N., Long B. J., Brodie A. M. H.: J. Med. Chem. *41*, 902 (1998).
9. Brodie A., Njar V. C. O.: US Patent: 5,994,335 (1999).
10. Grigoryev D. N., Long B. J., Nnane I. P., Njar V. C. O., Lui Y., Brodie A. M. H.: Brit. J. Cancer *81*, 622 (1999).
11. Nnane I. P., Njar V. C. O., Liu Y., Lu Q., Brodie A. M. H.: J. Steroid Biochem. Mol. Biol. *71*, 145 (1999).
12. Handratta D, H., Jelovac D., Long B. J., Kataria R., Nnane I. P., Njar V. C. O., Brodie A. M. H.: J. Med. Chem., in press.
13. Clement O. O., Freeman C. M., Hartmann R. W., Handratta V. D., Vasaitis T. S., Brodie A. M. H., Njar V. C. O.: J. Med. Chem., in press.

STEROIDAL CYCLOBUTANONES, SYNTHESIS AND TRANSFORMATIONS

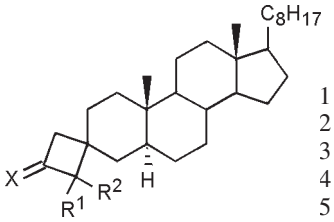
KRZYSZTOF BŁASZCZYK, HANNA KOENIG, KATARZYNA MEL, ZDZISŁAW PARYZEK*

Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland
E-mail: zparyzek@amu.edu.pl

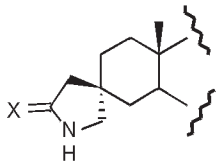
Cyclobutanone oximes rearrange under the Beckmann conditions to give γ-lactams[1].

In steroids, 5α-spiro[cholestane-3,1'-cyclobutane]-3'-one oximes are transformed to spiropyrrolidinones[3]. Stereospecificity of the process was clearly established[2]. In the case of α-substituted oximes (α-OH, -OR, -NR_2 groups for example), when stabilized cations are formed upon C(α) – C(sp^2) bond cleavage, abnormal Beckmann rearrangement has been observed[3]. However, the Beckmann rearrangement of α-chlorocyclobutanone oximes has not been reported.

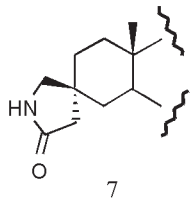
We prepared steroidal cyclobutanones from ketones in sequence of reactions involving cycloaddition of dichloroketene to the appropriate olefin. For example, 5α-cholestan-3--one was transformed *via* 3-methylene-5α-cholestane to spiro-cyclobutanone 1. It can be reduced to partially or fully dehalogenated products 2 and 3, respectively. The unsubstituted and α-chloro-cyclobutanones react with hydroxylamine to form oximes 4 and 5, respectively. Oximes of α,α-dichlorocyclobutanones have not been reported and our attempts to prepare oxime of the ketone 1 failed.



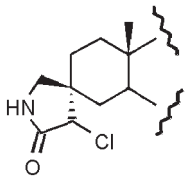
1 X = O, R^1 = R^2 = Cl
2 X = O, R^1 = H, R^2 = Cl
3 X = O, R^1 = R^2 = H
4 X = NOH, R^1 = R^2 = H
5 X = NOH, R^1 = H, R^2 = Cl



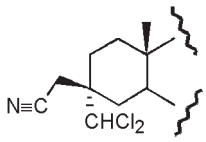
6



7



8



9

The α-unsubstituted oxime 4 rearranged upon treatment with thionyl chloride in benzene to the expected pyrrolidinones 6 and 7 in 62 % isolated yield[2]. However, the course of reaction was different, when cyclobutanone α-monochloro-oximes were subjected to similar reaction conditions. Thus, for example, reaction of 5 gave two compounds, which were isolated from the reaction mixture and separated by column chromatography. These were: the chloropyrolidinone 8 (11 % yield) and the nitrile 9 (30 % yield). This investigation was extended to rearrangements of few other steroidal α-chlorocyclobutanone oximes. In every reaction studied the Beckmann fission-displacement occurred and nitrile was found to be the more abundant component of the reaction mixture.

The reactions described are the first examples of the Beckmann rearrangement of alicyclic α-chloroximes resulting in simultaneous formation of normal and abnormal products. Analysis of the product formation and distribution permitted us to suggest a possible mechanism of the rearrangement.

Financial support of the work by the State Committee for Scientific Research (KBN, project No. 7 T09A 110 21) is gratefully acknowledged.

REFERENCES

1. (a) Jeffs P. W., Molina G., Cortese N. A., Hauck P. R., Wolfram J.: J. Org. Chem. *47*, 3876 (1982); (b) Greene

A. E., Depres J. P., Meana M. C., Crabbe P.: Tetrahedron Lett. *17*, 3755 (1976).
2. Błaszczyk K., Paryzek Z.: Liebigs Ann. Chem. *1993*, 1105; Nebois P., Greene A. E.: J. Org. Chem. *61*, 5210 (1996).
3. (a) Smith P. A. S., in: *Molecular Rearrangements* (de Mayo P., ed.), Chap. 8 and references cited therein. Interscience Publishers, New York 1963; (b) Hassner A., Nash E. G.: Tetrahedron Lett. *6*, 525 (1965); (c) Fráter G., Müller U., Günther W.: Tetrahedron Lett. *25*, 1133 (1984).

SCUTEPARVIN, A NEW NEOCLERODANE DITERPENOID FROM *Scutellaria parvula*

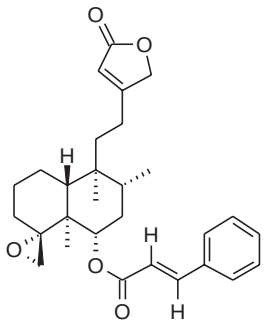
MAURIZIO BRUNO[a*], SERGIO ROSSELLI[a], ANTONELLA MAGGIO[a], FRANCO PIOZZI[a], LEONARDO SCAGLIONI[b], ORIETTA SERVETTAZ[c]

[a]Dipartimento di Chimica Organica "E. Paternò", Università di Palermo, Viale delle Scienze, Parco d'Orleans II, 90128, Palermo, [b]Dipartimento di Scienze Agroalimentari, Università di Milano, [c]Dipartimento di Biologia, Università di Milano, Italy
E-mail: bruno@dicpm.unipa.it

The genus *Scutellaria*, Lamiaceae (Labiatae) family, occurs with some 360 species spread throughout the world[1]. It is rich with neoclerodane diterpenoids, that usually show some heterocyclic functions: epoxides, lactones, hydrofurans groups. Many of these products have a remarkable antifeedant activity against pest insects. The chemistry of the diterpenoids from *Scutellaria* was recently reviewed[2].

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

From its aerial parts we isolated a new neoclerodane diterpenoid, scuteparvin (1). Its structure is rather similar to those of ajugarin V, isolated by Kubo[3] from *Ajuga remota*, the only difference being the occurrence of the *trans*-cinnamoyloxy group instead of an acetoxy on C-6.



1

REFERENCES

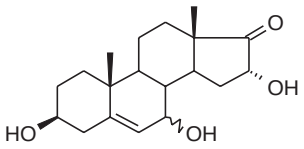
1. Willis J. C.: *A Dictionary of the Flowering Plants and Ferns*. Cambridge University Press, Cambridge 1966.
2. Bruno M., Piozzi F., Rosselli S.: Nat. Prod. Rep. *19*, 357 (2002).
3. Kubo I., Fukuyama Y., Chapya A.: Chem. Lett. *1983*, 223.

SYNTHESIS OF 7,16-DIHYDROXYDEHYDROEPIANDROSTERONE AND DERIVED HAPTENS

VLADIMÍR POUZAR[a*], IVAN ČERNÝ[a], MARIE BIČÍKOVÁ[b], RICHARD HAMPL[b]

[a]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, [b]Institute of Endocrinology, Národní 8, 116 98 Prague 1, Czech Republic
E-mail: pouzar@uochb.cas.cz

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



Synthetic routes for this type of steroids started with DHEA, which is transformed initially to a corresponding isomer of 7-hydroxy-DHEA. Stereoselective introduction of 16α-hydroxy group was accomplished using two principal ways. The first approach used formation of suitably substituted enolates from 17-ketones, selective epoxidation of 16,17 double bond, and subsequent rearrangement into 16α-hydroxy-17-oxo moiety[1,2]. Second method used bromination of 17-ketone into 16α-position and then solvolysis in a mixture of *N*,*N*-dimethylformamide – water into 16α-hydroxy derivative[3]. Both these procedures were used also for a preparation of 16α-hydroxy derivative from 7-oxo-DHEA.

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

Supported by grant No. 203/03/0142 GA CR; a part of research project Z4 055 905 AS CR.

REFERENCES

1. Pouzar V., Černý I., Lapčík O., Hill M., Hampl R.: Steroids *68*, 149 (2003).
2. Reich I. L., Reich H. J., Kneer N., Lardy H.: Steroids *67*, 221 (2002).
3. Numazawa M., Nagaoka M.: J. Org. Chem. *47*, 4024 (1982).

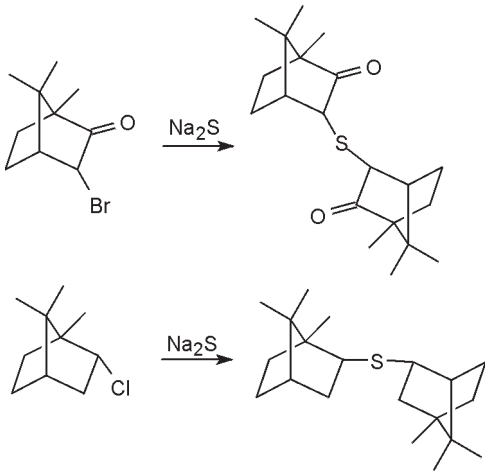
SYNTHESIS AND OXIDATION OF TERPENESULFIDES OF BORNANE STRUCTURE

SVETLANA A. RUBTSOVA, PAVEL N. KAZAKOV, ALEXANDER V. KUTCHIN

Institute of Chemistry, Komi Science Center, Ural Division of the Russian Academy of Sciences, 48 Str. Pervomaiskaya, 167982, Syktyvkar, Russian Federation
E-mail: rubtsova-sa@chemi.komisc.ru

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

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



Scheme 1

Earlier we shown high hemoselectivity of symmetrical and asymmetrical dialkyl-, alkylaryl-, diaryl-, dihalogendiaryl-, dibenzyl sulfides oxidation by chlorine dioxide[1]. The study of chlorine dioxide reaction ability in the reactions of synthesized terpenic sulfides oxidation is carried out in the present work. The reaction products are isolated and their structure is established by the methods of IR and NMR-spectroscopy.

This work is realized under financial support of Russian Foundation Basic Research (grant No. 01-03-96404 «Ural-2001»).

REFERENCES

1. Kutchin A., Rubtsova S., Loginova I.: Russ. Chem. Bull., Int. Ed. *2001*, 432.

LAPACHOL, DERIVATIVES AND ANALOGUES: ADDITIONAL PHARMACOLOGICAL ACTIVITIES AGAINST TROPICAL DISEASES

ANTÔNIO E. G. SANTANA[a], NADJA M. F. LIMA[a], CLARIANE S. CORREIA[a], LUCIANA A. SILVA[a], MARÍLIA O. F. GOULART[a], FERNANDO MENDONÇA[a], LEONOR L. LEON[b]

[a]Departamento de Quimica, CCEN, Universidade Federal de Alagoas, Maceio, Alagoas, 57072-970, [b]Departamento de Imunologia, FIOCRUZ, Rio de Janeiro, 21045-900, Brazil

Leishmaniasis is one of the major infectious diseases affecting the poorest regions of the world. Human infections with *Leishmania* protozoan parasites, transmitted *via* the bite of a sand fly, cause visceral, cutaneous or mucocutaneous leishmaniasis[1]. The global burden of leishmaniasis has remained stable for some years, causing 2.4 million disability adjusted life years lost and 59 000 deaths in 2001 (ref.[2]). Drug treatment today, besides the pentavalent antimonials, is restricted to a limited number of clinically useful drugs like pentamidine-isethionate or amphotericin B[1]. General treatment is unaffordable for many afflicted countries revealing an urgent need for new, safer and cheaper drugs[1].

Dengue viruses, single stranded RNA viruses of the family Flaviviridae, are the most common cause of arboviral disease in the world. They are found virtually throughout the tropics and cause an estimated 50–100 million illnesses annually, including 250 000–500 000 cases of dengue haemorrhagic fever – a severe manifestation of dengue – and 24 000 deaths. More than two fifths of the world's population (2.5 billion) live in areas potentially at risk for dengue. The incidence, distribution, and clinical severity of dengue has increased dramatically in the last years[3].

Numerous quinones play vital roles in the biochemistry of living cells and exert relevant biological activities. More especifically, hydroxyquinones had been recognised as potential lead structures against *Leishmania*[4] and natural lapachol isomers, isolated from *Calceolaria andina* as a new class of insecticides[5]. Naphthoquinones were also reported as larvicidal compounds[6].

One strategy to discover new drugs leads is to investigate classes of compounds potentially bioactive or old active compounds for newer uses. Therefore, the aim of the present study was to further investigate the natural isoprenic hydroxyquinone lapachol, isomers and derivatives (Fig. 1) toward their leishmanicidal activity against *Leishmania brasiliensis* and *L. amazonensis* and, against *Aedes aegypti*. Brine shrimp lethality assay (*Artemia salina*) (BSLA) was also employed, to look for a possible correlation on the different screened biological activities[7].

The bioassays followed the established protocols. *Leishmania amazonensis* (MHOM/BR/77/LTB0016 strain) promastigotes were grown at 26 °C in Schneider's *Drosophila* medium supplemented with 10 % (v/v) heat-inactivated fetal calf serum (FCS), with a pH value of 7.2. *L. braziliensis* (MCAN/BR/98/R619) promastigotes were grown in the same medium, same temperature and pH, but supplemented with 20 % FCS

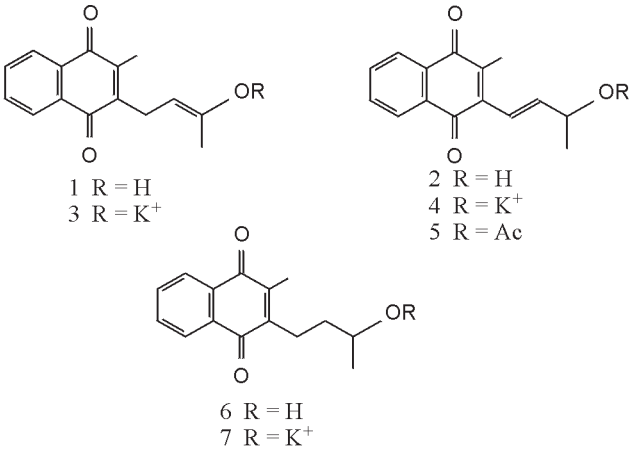


Fig. 1. Studied hydroxynaphthoquinones and derivatives

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

The results of the bioassays are presented in Table I. IC_{50}: values indicate the effective concentration of a compound in $mg.ml^{-1}$ necessary to achieve 50 % growth inhibition.

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

Table I
Antileishmanial activity (IC_{50}, $\mu g.ml^{-1}$) against *L. amazonensis* (MHOM/BR/77/LTB0016) and *L. braziliensis* (MCAN/BR/98/R619 and, toxicity against *Artemia salina* in $\mu g.ml^{-1}$, of the assayed hydroxyquinones and derivatives

Compounds	*L. amazonensis*	*L. braziliensis*	*Artemia salina*
Lapachol (1)	5.2±0.7	11.9±6.9	12.75 [6]
Isolapachol (2)	4.4±2.9	9.3±2.7	6.62
Lapachol, K^+ salt (3)	7.7±4.1	21.4±2.9	79.76
Isolapachol, K^+ salt (4)	7.8±0.2	15.8±0.0	0.21
Acetylisolapachol (5)	1.6±0.0	3.4±0.5	1.94
Dihydrolapachol (6)	7.3±0.3	54.0±9.0	23.56
Dihydrolapachol, K^+ salt (7)	ND	ND	33.19
Pentamidine Isethionate	0.28±0.05	11.6±1.6	ND

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

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

REFERENCES

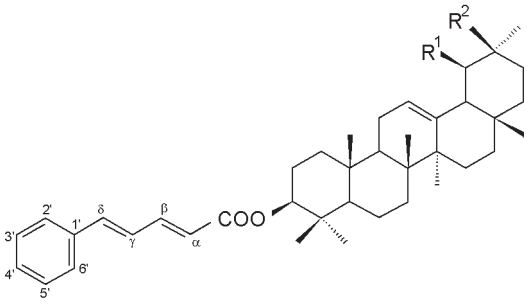
1. Davies C. R., Kaye P., Croft S. L. Sundar S.: Brit. Med. J. *326*, 377 (2003).
2. World Health Organization. Annex 3.: Burden of disease in DALYs by cause, sex and mortality stratum in WHO regions, estimates for 2001. In: The world health report. Geneva: WHO, 192 (2002).
3. Gibbons R. V., Vaughn D. W.: Brit. Med. J. *321*, 1563 (2002).
4. Kayser O., Kiderlen A. F., Laatsch H., Croft S. L.: Acta Trop. *77*, 307 (2000).
5. Khambay B. P. S., Jewess P.: Crop Protect. *19*, 597 (2000).
6. Ioset J.R., Marston A., Gupta M. P., Hostettmann K.: Phytochemistry *53*, 613 (2000).
7. Zani B., Chaves P. P. G., Queiroz R., De Oliveira A. B., Cardoso J. E., Anjos A. M. G., Grandi T. S. M.: Phytomedicine *2*, 47 (1995).
8. Shamsuzzaman S. M., Furuya M., Korenaga M., Imamura K., Hashiguchi Y.: Ann. Trop. Med. Parasitol. *93*, 613 (1999).
9. World Health Organization. Instructions for determining the susceptibility of resistance of mosquito larvae to insecticides, WHO/VBC 81-807, 1 (1981).
10. Vanhaecke P., Persoone G., Claus C., Sorgeloos P.: Ecotoxicol. Environ. Saf. *5*, 382 (1981).

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

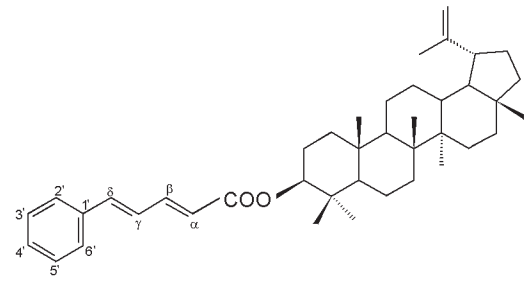
ANTÔNIO E. G. SANT'ANA, MARIA CLEONICE B. S. NASCIMENTO, MARGARIDA M. S. HUMBERTO

Departamento de Química, Universidade Federal de Alagoas, 57.072-970 Maceió-AL, Brazil
E-mail: aegs@qui.ufal.br

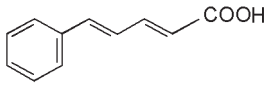
Peltastes peltatus (VELL.) WOODSON (Apocynaceae) is a creeper widely spread over in the southern states of Brazil.



	R^1	R^2
1	H	Me
2	Me	H



3



4

Species belonging to the genus *Peltastes* are rarely considered in the chemical literature and no report concerning the chemistry is available. The mutagenic activity of the aqueous extract of *P. peltatus* has been recently reported[1]. The aim of the present work was to investigate the chemical composition of *P. peltatus*. Plant material was collected in Alagoas state (Brazil) and voucher specimen (number 3268) has been deposited in the herbarium of the Department of Botany of the University of Brasília.

The air-dried and powdered stem (2.7 kg) of *P. peltatus* was extracted in a Soxhlet apparatus with EtOH (10 L) to yield 185 g of crude extract. After suspension in MeOH/H_2O (3:2) solution and extraction with *n*-hexane, chloroform and ethyl acetate (2.0 L of each solvent) we obtained the hexane, chloroform, ethyl acetate and aqueous fraction. Part of the hexane fraction (58.0 g) was chromatographed on silica gel column, eluted with *n*-hexane, gradually increasing the polarity with $CHCl_3$ to yield a solid material (9.5 g), which, on TLC, gave a positive Liebermann-Burchard test and a violet color with ceric sulphate, suggesting a triterpenic nature. Rechromatography on a silica gel column yielded compound 1 as a crystalline solid (3.1 g) a 2:1 mixture of 1 and 2 (3.2 g), and 3 as an amorphous material (0.8 g). The structures of these compounds were established by MS, 1D- and 2D-NMR experiments as well as by chemical degradation.

The molecular formula of compound 1 was assigned as $C_{41}H_{58}O_2$ based on elemental analysis, ^{1}H and ^{13}C NMR and EIMS (M^+, *m*/*z* 582). Its IR spectrum showed absorption bands at 1708 and 1242 cm^{-1}, while its UV spectrum showed absorption maxima at 247 and 308 nm (log ε = 4.39; 4.48, MeOH), related to an aromatic system conjugated with a diene (König and Rimpler, 1985). The ^{1}H NMR spectrum of 1 revealed the presence of eight tertiary methyl groups (δ_H 0.83, 0.87, 0.88, 0.91, 0.93, 0.98, 0.99 and 1.14) and one olefinic hydrogen (δ_H 5.18, bt, *J* = 3.4 Hz), typical of β-amyrin. This was also supported by the observation of a base ion peak at *m*/*z* 218 corresponding to the ion resultant from the reverse Diels-Alder fragmentation characteristic of the derivatives of Δ^{12}-oleanene/ursene.

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

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

Peltastine B (3) was isolated as a white amorphous solid, m.p. 170–172 °C (EtOH). The ^{1}H and ^{13}C NMR spectra of 3 were closely related to those of compound 1, except for the triterpenoid moiety. Its molecular formula $C_{41}H_{58}O_2$ was established by EIMS (M^+ *m*/*z* = 582) in combination with ^{1}H and ^{13}C NMR, indicating that 3 was isomeric with compounds 1 and 2. The presence of an ester linkage in 3 was indicated by the carbonyl signal at δ_C 167.1 together with IR absorption at 1709 cm^{-1}.

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

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

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

REFERENCES

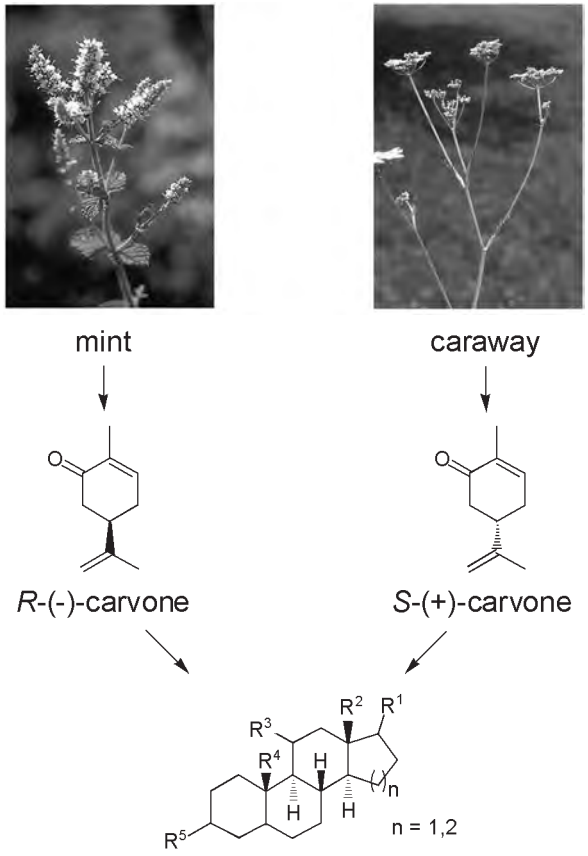
1. Horn R. C., Vargas V. M. F.: Mutagenesis *18*, 113 (2003).
2. König G., Rimpler H.: Phytochemistry *24*, 1245 (1985).
3. Marato S. B., Kundu A. P.: Phytochemistry *37*, 1517 (1994).
4. Ahmad V. U., Bano S., Mohammad F. V.: Planta Med. *6*, 521 (1985).
5. Domínguez X. A., Marroquín J., Olguín L. M., Morales F., Valdez V.: Phytochemistry *13*, 2617 (1974).

CARVONE AS A STARTING MATERIAL FOR THE TOTAL SYNTHESIS OF STEROIDS

FLORENCE C. E. SARABÈR[a], SVETLANA V. DRACH[b], TATYANA V. CHARNIKHOVA[b], ALEXANDER V. BARANOVSKI[b], BEN J. M. JANSEN[a], AEDE DE GROOT[a]

[a]Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands, [b]Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich Str. 5/2, 220141 Minsk, Belarus
E-mail: floor.saraber@wur.nl, http://www.ftns.wau.nl/oc/

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



A route toward C,D-*cis* coupled steroid skeletons, in which the use of carvone leads to an enantiomerically pure D-homosteroid skeleton, has already been developed and has been accepted for publication[1].

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

REFERENCES

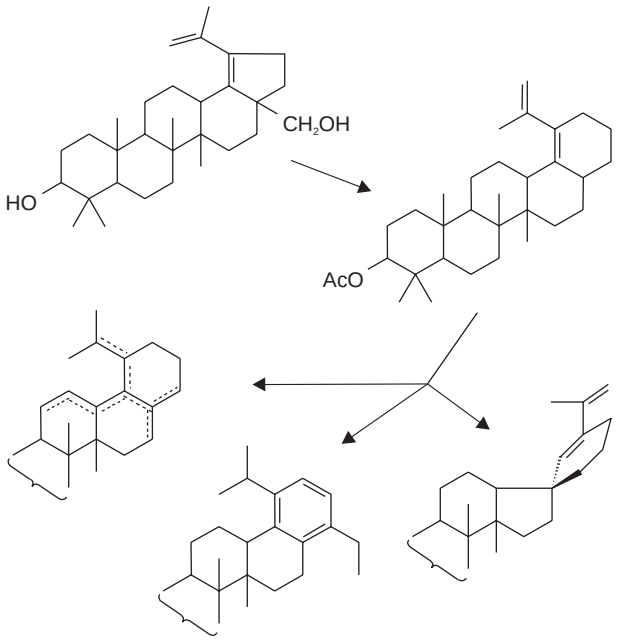
1. Drach S., Charnikhova T., Sarabèr F., Jansen B., de Groot A.: Tetrahedron (2003), in press.

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

JAN SEJBAL, VÁCLAV VANĚK

Department of Organic Chemistry, Charles University, Albertov 6, 128 40 Prague 2, Czech Republic
E-mail: sejbal@natur.cuni.cz

Triterpenoids, especially lupane derivatives are subject of intensive biological studies in the last decade because of their anti-cancer and anti-HIV activities. Among biologically active triterpenoids are commonly present lupane derivatives



bearing more oxygen containing functional groups or degraded triterpenois.

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

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

CONSTITUENTS AND BIOLOGICAL ACTIVITY OF THE ESSENTIAL OILS FROM *Sideritis italica* (MILLER) GREUTER ET BURDET (LAMIACEAE)

FELICE SENATORE[a], MAURIZIO BRUNO[b], FRANCO PIOZZI[b]

[a]Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II" – Via D. Montesano, 49-I 80131 Napoli, [b]Dipartimento di Chimica Organica, Università degli Studi di Palermo, Viale delle Scienze, Parco d'Orleans II-I 90128 Palermo, Italy
E-mail: fesenato@unina.it

The genus *Sideritis* (Lamiaceae) counts at least 150 species[1] occurring in temperate and tropical regions of the northern hemisphere; the countries around the Mediterranean Sea are particularly rich. Several species are widely used in the folk medicine of many countries for their anti-inflammatory, antispasmodic, carminative, sedative, antitussive, stomachic, anticonvulsant and antifeedant activities. The essential oils are used for many therapeutic purposes, for instance as pulmonary disinfectants, diuretics, stomachics, neurorelaxing[2]. The aerial parts of *Sideritis* taxa have been largely investigated and diterpenoids were the main metabolites isolated from them[3]. On the other hand, many papers on the composition of the essential oils have been published[4–6] and antimicrobial and analgesic activities have been pointed out[7,8]. The species *Sideritis italica* occurs in Sicily and in Southern Italy[9] and previous papers reported the occurrence of several *ent*-kaurane diterpenoids in the aerial parts. The present is the first paper on the chemical composition of the essential oil from leaves and flower heads of this plant. Oils were extracted and examined following the usual procedure[10]. The yields of the essential oils from flower heads and leaves of *S. italica* were 0.16 % and 0.14 %, respectively. 49 compounds in the flower heads and 31 compounds in the leaves, amounting to 93.7 % and 92.4 % of the essential oil, were identified. In the oil from flower heads kaur-15-ene and β-cubebene were the main components representing one third of the oil. *p*-Methoxyacetophenone was the major component of the oil from leaves. The results of the studies on the allelopathic activity of the oils put in evidence an inhibiting action, *in vitro*, as on the percentage of germination of the seeds as on the development immediately following to the germination of the seed (to the light) of *Raphanus sativus*.

REFERENCES

1. Willis J. C.: *Dictionary of the Flowering Plants and Ferns*, 7th Ed., pp. 10–38. Cambridge University Press, Cambridge 1966.
2. Senatore F.: *Oli Essenziali. Origine, estrazione ed analisi chimica*. EMSI–Roma, Roma 2000.
3. Gonzalez A. G., Luis J. G., Ravelo A. G.: *Plantas Iberoamericanas Fuentes de Moleculas Bioactivas*, Vol. 2, pp. 125–183. Ed. Romero, Tenerife 1990.
4. Laeer U., Glombitza K. W., Neugebauer M.: Planta Med. *62*, 81 (1996).
5. Kirimer N., Tabanca N., Ozek T., Basher K. H. C., Tumen G.: Chem. Nat. Compd. *35*, 61 (1999).
6. Galati E. M., Germano M. P., Rossitto A., Tzakou O., Skaltsa H., Roussis V.: J. Essent. Oil Res. *8*, 303 (1996).
7. Gergis V., Spiliotis V., Argyriadou N., Poulos C.: Flavour Fragrance J. *6*, 93 (1991).
8. Ezer N., Abbasoglu U.: Fitoterapia *67*, 474 (1996).
9. Greuter W.: *Med-Checklist*, Vol. 3, p. 350. Ed. Conserv. Jard. Botan., Genève 1986.
10. Senatore F., Bruno M.: Flavour Fragrance J. *18*, 195 (2003).

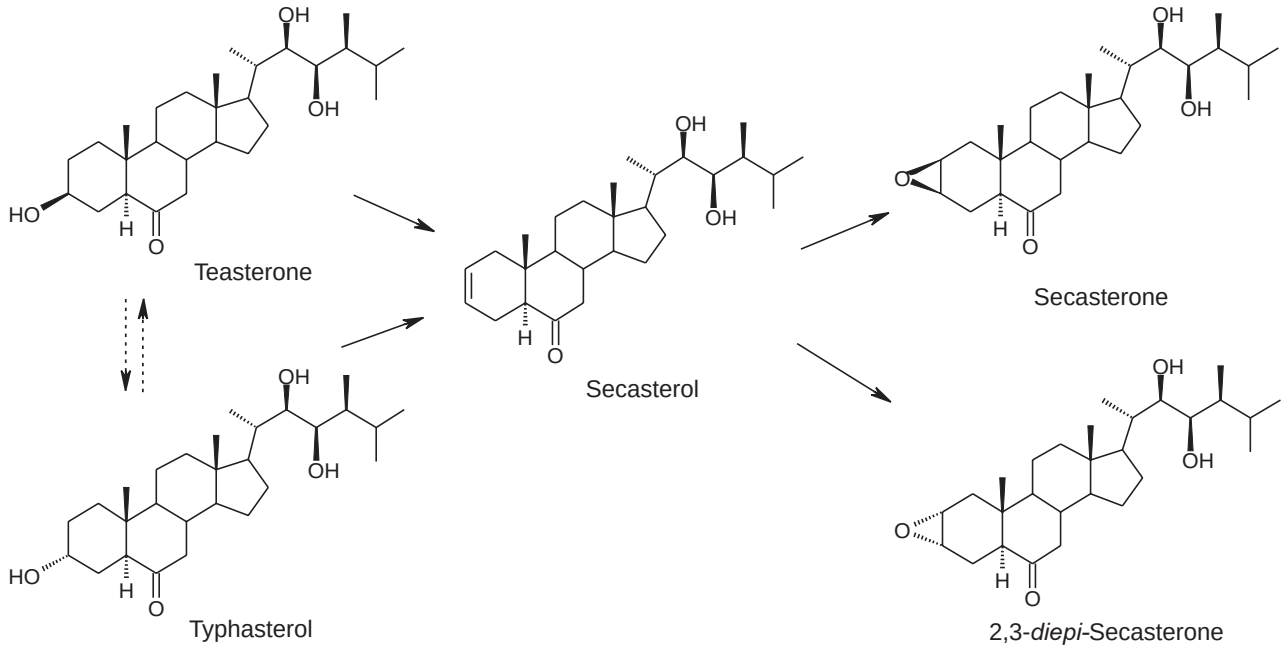
BIOSYNTHESIS OF 2,3-EPOXYBRASSINOSTEROIDS IN RYE SEEDLINGS

ANDREY ANTONCHICK[a,b], BERND SCHNEIDER[a], OLGA V. KONSTANTINOVA[b], VLADIMIR N. ZHABINSKII[b], VLADIMIR A. KHRIPACH[b]

[a]Max-Planck-Institute for Chemical Ecology, Beutenberg-Campus, Winzerlaer Str. 10, 07745 Jena, Germany, [b]Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich Str. 5/2, 220141 Minsk, Belarus
E-mail: schneider@ice.mpg.de

2,3-Epoxybrassinosteroids such as secasterone and 24-*epi*-secasterone previously have been reported in seeds of *Secale cereale*[1] and *Lychnis viscaria*[2], respectively. We analyzed seedlings of two rye varieties (*Secale cereale*, cv. "Sorom" and cv. "Petka") for the occurrence of brassinosteroids with special emphasis on 2,3-epoxybrassinosteroids and putative biosynthetic precursors. Secasterone and 2,3-*diepi*-secasterone, which is reported in this study for the first time as a naturally occurring compound, were found in rye seedlings. In addition, secasterol, the first 2,3-olefinic brassinosteroid has been identified from the same plant source[3]. Secasterone and 2,3-*diepi*-secasterone were synthesized as deuterated analytical standards[4], and deuterated teasterone, typhasterol, and secasterol were synthesized as putative biosynthetic precursors. Feeding experiments using deuterated intermediates were carried out in rye seedlings (*Secale cereale*) in order to investigate biosynthetic incorporation into 2,3-epoxybrassinosteroids. Biosynthetic products were identified by GC-MS-SIM analysis.

Deuterated teasterone, and typhasterol, upon administration to rye seedlings, were converted to secasterol, secasterone and 2,3-*diepi*-secasterone. Additional feeding experiments showed a high conversion rate of secasterol to both 2,3-epoxybrassinosteroids. From these findings, the biosynthetic se-



quence teasterone/typhasterol to secasterol to secasterone and 2,3-*diepi*-secasterone has been demonstrated to be operative in *Secale cereale*[3].

REFERENCES

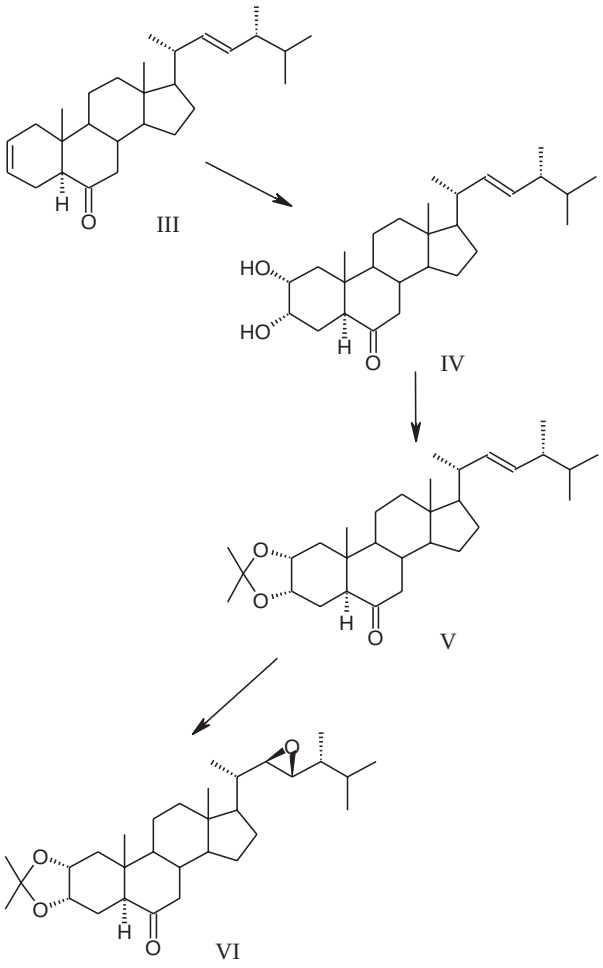
1. Schmidt J., Spengler B., Yokota T., Nakayama M., Takatsuto S., Voigt B., Adam G.: Phytochemistry *38*, 1095 (1995).
2. Friebe A., Volz A., Schmidt J., Voigt B., Adam G., Schnabl H.: Phytochemistry *52*, 1607 (1999).
3. Antonchick A., Schneider B., Konstantinova O. V., Zhabinskii V. N., Khripach V. A.: Phytochemistry (2003), in press.
4. Khripach V. A., Zhabinskii V. N., Konstantinova O. V., Antonchick A. P., Schneider B.: Steroids *67*, 587 (2002).

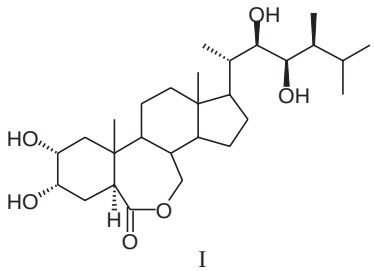
SYNTHESIS OF A NEW BRASSINOLIDE ANALOG AND ITS BIOASSAY

MIROSLAV ŠÍŠA[a], SONIA ESPELTA[b], CARME BROSA[b], LADISLAV KOHOUT[a]

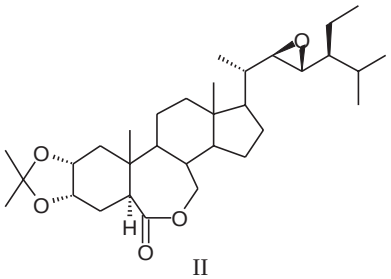
[a]Institute of Organic Chemistry and Biochemistry, Academy of Scineces of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic, [b]Institut Químic de Sarria, Universitat Ramon Llull, Via Augusta 390, 08024 Barcelona, Spain E-mail: sisa@uochb.cas.cz, kohout@uochb.cas.cz, brosa@iqs.url.es

Brassinolide I is known as the most potent compound for plant growth promoting activity[1]. It was reported[2] that brassinolide analog II showed even better activity under the field conditions, although it was completely inactive under the rice lamina inclination test. In our structure-activity relationships studies of brassinosteroids[3] we prepared brassinolide analog VI, which shows better activity already in the rice lamina inclination test in comparison with brassinosteroid II.





I



II

Synthesis of our brassinosteroids started from (22*E*)-5α--ergosta-2,22-dien-6-one (III), which was carefully hydroxylated by osmium tetroxide for 2 hours and the acquired (22*E*)--2α,3α-dihydroxy-5α-ergosta-2,22-dien-6-one (IV) was transformed to its acetonide (V). Subsequent epoxidation with 3-chloro-perbenzoic acid gave compound VI.

This work was supported by grants Nos. 203/01/0083, IBS 4055304, A 4055204 and Z 4055905.

REFERENCES

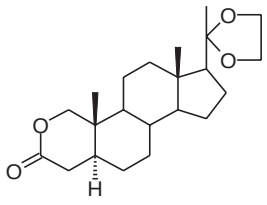
1. Sasse J.: *Physiological Actions of Brassinosteroids*, Chap. 7., in *Brassinosteroids: Steroidal Plant Hormones* (Sakurai A., Yokota T., Clouse S. D., ed.), pp. 137–161. Springer-Verlag, Tokyo 1999.
2. Kakiuchi T., Kamuro Y., Takatsuto S., Kobayashi K.: Agric. Biol. Chem. *52*, 2381 (1988).
3. Strnad M., Kohout L.: Plant Growth Regul. *40*, 39 (2003).

DIBAL REDUCTION OF SOME STEROIDAL LACTONES

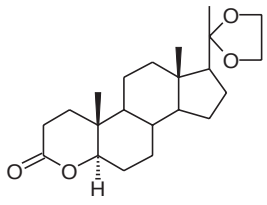
BARBORA SLAVÍKOVÁ, ALEXANDER KASAL

Institute of Organic Chemistry and Biochemistry, Fleminovo n. 2, 166 10 Prague 6
E-mail: slavikova@uochb.cas.cz

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



1



2

This work was supported by grants No. S5011007 and Z4 055 905.

REFERENCES

1. Pappo R., Jung C. J.: Tetrahedron Lett. *1962*, 365.
2. Pettit G. R., Kasturi T. R.: J. Org. Chem. *26*, 4557 (1961).
3. Suginome H., Yamada S.: Bull. Chem. Soc. Jpn. *60*, 2453 (1987).

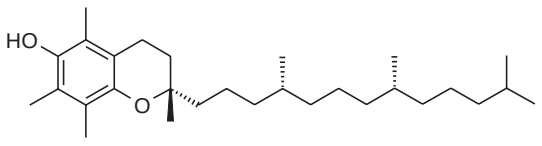
ENANTIOSELECTIVE SYNTHESIS OF (2*R*,6*R*)-2,6,10-TRIMETHYLUNDECANE-1-OL – CHIRAL PRECURSOR FOR THE PREPARATION OF NATURAL VITAMIN E

ANNA YU. SPIVAK, GUL'NARA A. EMEL'YANOVA, MARINA I. MALLYABAEVA, VICTOR N. ODINOKOV

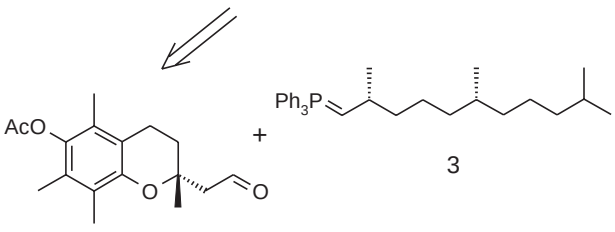
Institute of Petrochemistry and Catalysis, Bashkortostan Republic Academy of Sciences and the Ufa Scientific Centre of the Russian Academy of Sciences, 141 prosp. Oktyabrya, 450075 Ufa, Russian Federation
E-mail: ink@anrb.ru

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

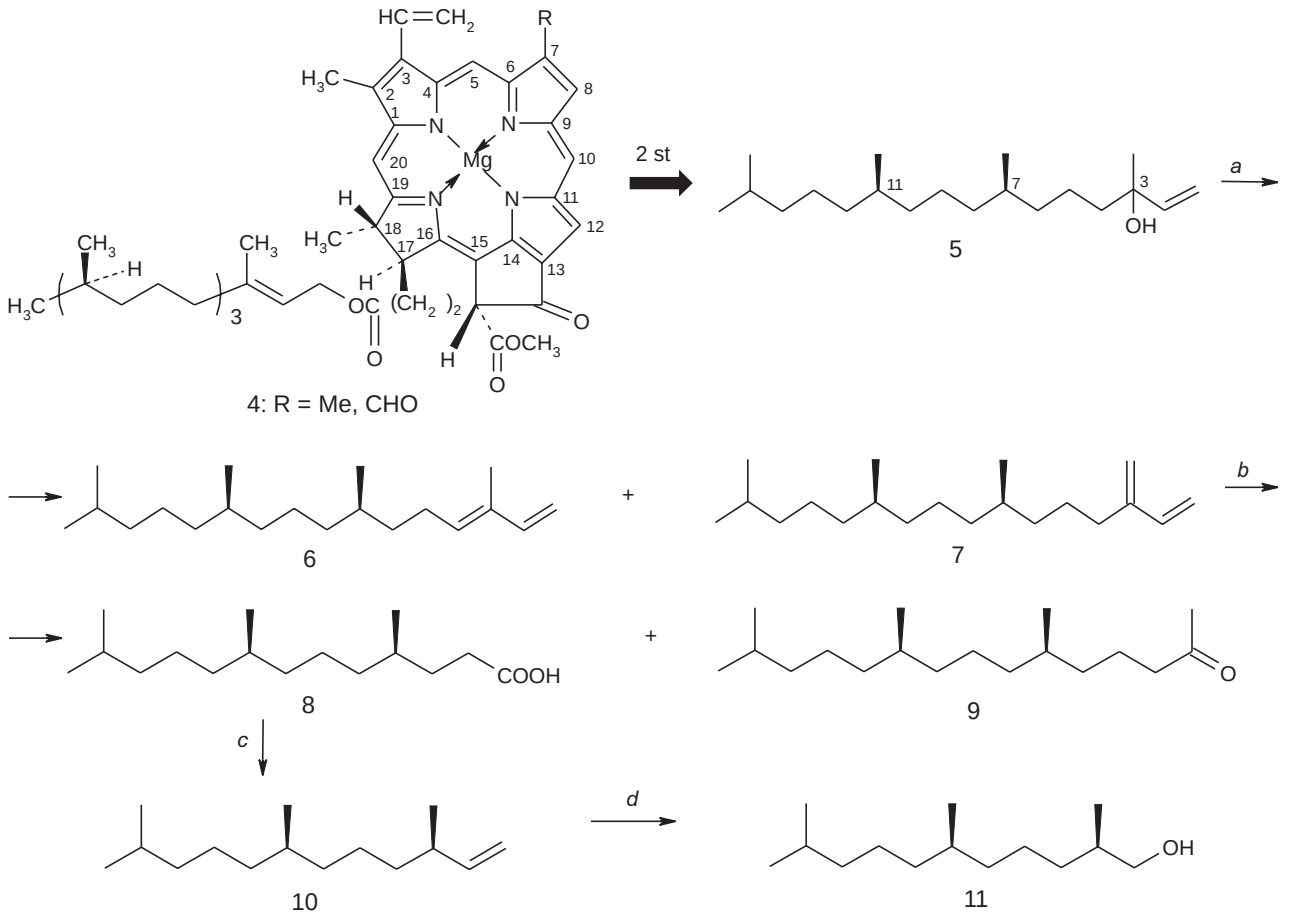
We developed the effective enantiospecific synthesis of alcohol 11 based on the dehydration of (3*RS*,7*R*,11*R*)-isophy-



1



2 3



a: TsOH-SiO_2/PhH, r.t., 1 h; *b*: O_3/$Ba(OH)_2$/Me_2CO, $KMnO_4$/H_2SO_4/H_2O; *c*: $Pb(OAc)_4$/$Cu(OAc)_2.H_2O$/Py, PhH; *d*: O_3/CH_2Cl_2-MeOH (5:1), $NaBH_4$/MeOH

tol (5) obtained *via* the ozonolysis of chlorophyll (4) followed by the interaction of *R*,*R*-phytone formed with vinylmagnesium bromide[2]. It was found, that the reaction of TsOH-SiO_2 with isophytol 5 under the mild conditions resulted in the mixture of phytadiens 6 (a mixture of *E*/*Z*-isomers 1:3) and 7 in a ratio 6:1 in a quantitative yield. Ozonolysis of the diene mixture 6, 7 and the further oxidation of the ozonolysis products with $KMnO_4$ led to the easily separated mixture of (4*R*,8*R*)-4,8,12-trimethyltridecane acid 8 and phytone (9). The oxidative decarboxylation of the acid 8 yielded (3*R*,7*R*)--3,7,11-dodec-1-ene (10), which ozonolysis followed by the reaction with $NaBH_4$ gave the target alcohol 11.

REFERENCES

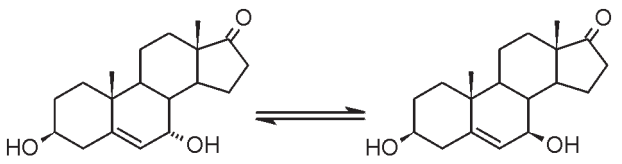
1. Odinokov V. N., Spivak A. Yu., Emelyanova G. A., Gamalevich G. D., Serebryakov E. P.: Izv. Akad. Nauk, Ser. Khim. *2001*, 2026.
2. Odinokov V. N., Mallyabaeva M. I., Spivak A. Yu., Emelyanova G. A., Dzhemilev U. M.: Dokl. Akad. Nauk *380*, 201 (2001).

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

EVA ŠŤASTNÁ, VLADIMÍR POUZAR, HANA CHODOUNSKÁ*

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: hchod@uochb.cas.cz

7-Hydroxylation of 3β-hydroxyandrost-5-ene steroids is a reaction occuring in many mammalian tissues[1]. Hydroxylation of 3β-hydroxyandrost-5-en-17-one (DHEA) with rat liver homogenate at 7α-position and subsequent conversion to othre 7-oxygenated steroids in the sequence DHEA →



7α–OH 7β–OH

7α-hydroxy-DHEA → 7-oxo-DHEA → 7β-hydroxy-DHEA was described[2].

These 7α-OH and 7β-OH isomers have beneficial biological effects[3] (e.g. on immune system) but their activity is different. Therefore, it seems to be useful to prove the stability of both isomers under different conditions.

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

Supported by research project Z4 055 905 and grant No. IAA4055305 of the Grant Agency of Academy of Sciences of the Czech Republic.

REFERENCES

1. Hampl R., Morfin R., Stárka L.: Endocr. Reg. *31*, 211 (1997).
2. Lardy H., Marwah A., Marwah P.: Lipids *37*, 1187 (2002).
3. Morfin R., Courchay G.: J. Steroid Biochem. Mol. Biol. *50*, 91 (1994).

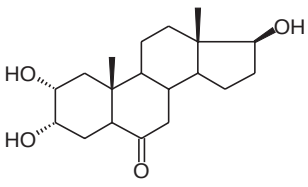
SIMPLE BRASSINOLIDE ANALOGUES EXHIBITING TYPICAL BRASSINOLIDE ACTIVITY

MIROSLAV STRNAD[a], LADISLAV KOHOUT[b]

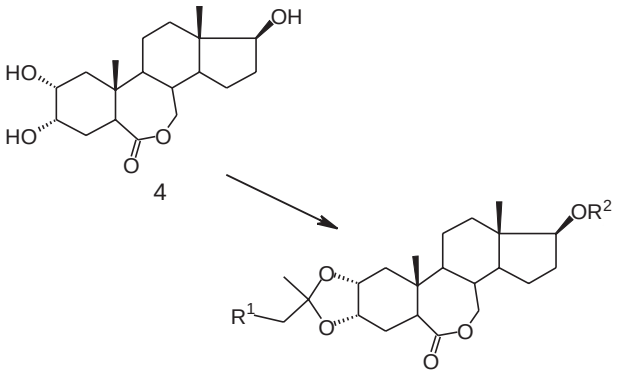
[a]Laboratory of Growth Regulators, Faculty of Sciences of the Palacký University & Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Šlechtitelů 11, 783 71 Olomouc, [b]Department of Steroid Chemistry, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: strnad@aix.upol.cz

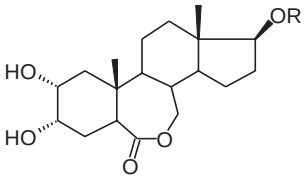
A new synthetic brassinolide analogue, 2α,3α-dihydroxy-17β-(3-methylbutyryloxy)-7-oxa-7a-homo-5α-androstan-6-one (11), has been shown to exhibit typical brassinolide activity – splitting of the bean second internode. It was prepared from the known lactone 2α,3α,17β-trihydroxy-7-oxa-7a-homo-5α-androstan-6-one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

However, in the second bean internode bioassay 100 times more 10 or 11 compared to 24-epibrassinolide is required to obtain the same effects. Analogues with β-oriented hydroxyl groups at C-2 and C-3 (14, 15), a 6-ketone (17, 18) or 6-oxa-7-oxo-lactone system (12, 13), in ring B lack the typical brassinolide activity. In addition, the active brassinosteroids applied to the second internode stimulated a similar, but 30 % lower elongation of the first internode. From data presented here we conclude that the presence of two hydroxy groups in



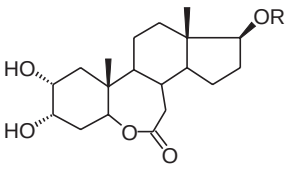
3



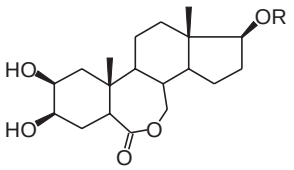


10, 11

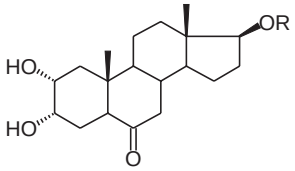
5 R^1 = H, R^2 = H
6 R^1 = H, R^2 = 2-MeBut
7 R^1 = H, R^2 = 3-MeBut
8 R^1 = CH_3, R^2 = H
9 R^1 = CH_3, R^2 = 3-MeBut
10 R = 2-MeBut
11 R = 3-MeBut



12 R = 2-MeBut
13 R = 3-MeBut



14 R = 2-MeBut
15 R = 3-MeBut



17 R = 2-MeBut
18 R = 3-MeBut

2-MeBut = 2-methyl-butyryl
3-MeBut = 3-methyl-butyryl

the positions 22 and 23 of the brassinolide side chain, which are considered as a key structural requirement, is not absolutely necessary for a compound to exhibit typical brassinosteroid activity. Nevertheless, these compounds have generally 2–10 times lower activity than that having 22,23-vicinal diol in the side chain.

IMMUNOANALYTICAL SYSTEM FOR DETERMINATION OF BRASSINOSTEROIDS IN PLANT TISSUES

J. SWACZYNOVÁ[a], M. ŠÍŠA[b], L. KOHOUT[b], M. STRNAD[a]

[a]Laboratory of Growth Regulators, Institute of Experimental Botany, Academy of Sciences of the Czech Republic and Palacký University, Šlechtitelů 11, 783 71 Olomouc, [b]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: swaczynova@prfholnt.upol.cz

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

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

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

NEO-CLERODANE DITERPENES FROM *Ajuga remota* BENTH. (LABIATAE)

JOSEP COLL[*], YUDELSY TANDRON

Instituto de Investigaciones Químicas y Ambientales de Barcelona "Josep Pascual Vila", Consejo Superior de Investigaciones Científicas, Jordi Girona 18-26, 08034 Barcelona, Spain
E-mail: jctqob@iiqab.csic.es

Ajuga remota is a shrub growing widely in East Africa, and has been used in traditional medicine for the treatment of skin diseases, toothache, headache, fever, dysentery, high blood pressure, stomachache, malaria, edema, pneumonia and liver problems[1,2]. Results of tests for antifungal and antimalarial activities have been reported recently[3–5]. Following the observation that African armyworms did not attack *A. remota* leaves, clerodane diterpenes (ajugarin I, II, and III) were isolated as moderately strong antifeedants against *Spodoptera exempta* (AJG-I and II min conc 100 ppm) and *S. littoralis* (AJG-I and II min conc 300 ppm)[6]. Ajugarin IV was later isolated in trace amount, exhibiting insecticidal activity against *Bombyx mori* (at 500 ppm, LD_{95}) and growth inhibitory activity against pink bollworm *Pectinophora gossypiella* (at 500 ppm, ED_{50})[7]. Ajugarin V, an additional new diterpene isolated in trace amount, exhibited neither antifeedant nor insecticidal activity[8]. The isolation of clerodin (no data) as an antifeedant was also reported[9]. The whole plant *A. bracteosa* (syn. *A. remota*) afforded recently bracteonin A, 14,15-dihydroajugapitin, and 14-hydro-15-hydroxyajugapitin[10].

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

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

Dihydroclerodin (reported as a natural compound in *A. parviflora*[11]), a newly isolated compound from *A. remota*, was eluted at 31 min rt. The presence of a hexahydrofurofuran moiety in the molecule was derived from the chemical shifts in ^{1}H NMR [δ 4.07 (*dd*, *J* = 11.5, 5.5 Hz), 2.83 (*m*), 3.83 (2H, *m*), 5.61 ppm (*d*, *J* = 5.0 Hz); assigned to H_1-11, H_1-13, H_2-15 and H_1-16], as well as in ^{13}C NMR [δ 85.2, 33.4, 42.1, 32.7, 68.3 and 107.7]. Clerodin eluted at rt 36 min, and ajugapitin[12] also isolated for the first time from *A. remota* at 37 min. Dihydroajugapitin (reported previously in *A. chamaepitys*[13], *A. pseudoiva*[14], *A. iva*[15], and recently in *A. bracteosa*[10]) was eluted at 34 min rt.

In fractions eluting at 23–24 min the epimeric mixture of 15-hydroxydihydroclerodin (scutecyprol A) was also isolated. The corresponding mixture of 15-hydroxydihydroajugapitin[10,16] was also present but in trace amounts at 24–25 min. 15-Hydroxydihydroclerodanes have been reported mostly from the genus *Scutellaria*[17].

Financial support by MCYT grant (AGL2001/2285) and predoctoral fellowship (to YT) is gratefully acknowledged.

REFERENCES

1. Kokwaro J. O.: *Medicinal Plants of East Africa*. East Africa Lit Bureau/Kenya Lit. Bureau, Nairobi, Kenya. 106/118 (1976/1993 (2nd ed)); cf[2–4].
2. Odek-Ogunde M., Rajab M. S., Migwi G. J., Ndegwa J. M.: Planta Med. *59*, 573 (1993).
3. Kariba R. M.: Fitoterapia *72*, 177 (2001).
4. Kuria K. A. M., De Coster S., Muriuki G., Masengo W., Kibwage I., Hoogmartens J., Laekeman G. M.: J. Ethnopharmacol. *74* 141 (2001).
5. Kuria K. A. M., Chepkwony H., Govaerts C., Roets E., Busson R., De Witte P., Zupko I., Hoornaert G., Quirynen L., Maes L., Janssens L., Hoogmartens J., Laekeman G.: J. Nat. Prod. *65*, 789 (2002).
6. Kubo I., Lee Y.-W., Balogh-Nair V., Nakanishi K., Chapya A.: J. Chem. Soc., Chem. Commun. *1976*, 949.
7. Kubo I., Klocke J. A., Miura I., Fukuyama Y.: J. Chem. Soc., Chem. Commun. *1982*, 618.
8. Kubo I., Fukuyama Y., Chapya A.: Chem. Lett. *1983*, 223.
9. Kubo I., Kido M., Fukuyama Y.: J. Chem. Soc., Chem. Commun. *1980*, 897.
10. Verma V. H., Mahmood U., Singh B.: Nat. Prod. Lett. *16*, 255 (2002).
11. Beauchamp P. S., Bottini A. T., Caselles M. C., Dev V., Hope H., Larter M., Pant A. K., Raffel R. J., Sharma V. K., Wyatt D.: Phytochemistry *43*, 827 (1996).
12. Coll J.: Phytochem. Anal. *13*, 372 (2002).
13. Hernández A., Pascual C., Sanz J., Rodríguez B.: Phytochemistry *21,* 2909 (1982).
14. Camps F., Coll J., Dargallo, O.: Phytochemistry *23*, 387 (1984).
15. Bondí M. L., Al-Hillo M. R. Y., Lamara K., Ladjel S., Bruno M., Piozzi F., Simmonds M. S. J.: Biochem. Syst. Ecol. *28*, 1023 (2000).
16. Camps F., Coll J., Dargallo O.: Phytochemistry *23*, 2577 (1984).
17. Bruno M., Piozzi F., Rosselli S.: Nat. Prod. Rep. *19*, 357 (2002).

NEO-CLERODANE DITERPENES FROM *Teucrium fruticans* L. (LABIATAE)

JOSEP COLL*, YUDELSY TANDRON

Instituto de Investigaciones Químicas y Ambientales de Barcelona "Josep Pascual Vila", Consejo Superior de Investigaciones Científicas, Jordi Girona 18-26, 08034 Barcelona, Spain
E-mail: jctqob@iiqab.csic.es

The insect antifeedant property of clerodane diterpenes is the most extensively studied bioactivity of these compounds[1]. Various genera of the plant family Labiatae, namely *Scutellaria* and *Ajuga* are reported to produce some of the most potent clerodane antifeedants. In *Scutellaria*, jodrellin B (occurring in *S. albida*, *S. galericulata*, *S. grossa*, *S. polyodon*, and *S. woronowii*) and scutecyprol B (found in *S. columnae*, *S. cypria*, *S. grossa*, and *S. rubicunda*) show the highest antifeedant index against *Spodoptera littoralis*[2]. From *Ajuga pseudoiva* leaves, 14,15-dihydroajugapitin displayed the highest activity (AI > 90 even at 1 ppm dose) amongst several highly active compounds isolated[3].

The genus *Teucrium* (family Labiatae) is one of the richest sources of clerodane diterpenes, and the new natural products are conveniently reviewed periodically[4]. Most of the reported structures display a furan ring system in the side-chain, with an oxygen bearing C-12. The isolates from *T. fruticans*[5,6] fruticolone, isofruticolone and 8β-hydroxyfruticolone are exceptional for the absence of susbstitution at C-11 and C-12. Only a few other exceptions amongst more than 200 compounds are known[4].

Since *T. fruticans* is a widely distributed species and readily available, it was chosen to evaluate the HPLC behavior of furo-clerodanes. The diterpene fraction on HPLC analysis appeared more complex thananticipated. Thus, the isolation, structural elucidation and antifeedant activity of previously unreported neo-clerodanes, with different substitution on the decalin portion and in the side chain is here reported.

In previous studies[5–7] the structures of neo-clerodane diterpenoids isolated from *Teucrium fruticans* have been reported. A DCM extract of the aerial parts of this plant has now allowed the isolation of a new family of clerodanes present in the plant.

The wavelength of furo-diterpenes chromophore for UV detection is close to 210 nm, and since low sensitivity results, the analysis may be seriously interfered by other compounds with high UV absorption. Both the specificity of the method and detection sensitivity can be improved by adopting evaporative light scattering detector (ELSD) for compounds with low (or not at all) UV absorption.

HPLC coupled on-line to an ELSD provided data about the order of elution of the various furo-clerodanes present in T. fruticans extracts and a preliminary quantification. Semi-prep-HPLC proved to be a convenient purification procedure, occasionally being followed by TLC.

In addition to three of the reported neo-clerodanes found in this plant (fruticolone, isofruticolone, 8β-hydroxyfruticolone), 6-acetyl-teujaponin B (previously reported from *Teucrium scordium* and *Teucrium grisebachii*)[8,9] was also isolated from *T. fruticans*. The structures of new neo-clerodanes isolated in the present work were: 7β-hydroxyfruticolone, 11-hydroxyfruticolone and 10-hydroxy-6-acetyl-teujaponin B. Unambiguous structural elucidation was based on extensive spectral (one and two-dimensional NMR experiments) studies. The new compounds were assayed for their antifeedant activity against *Spodoptera littoralis*.

Financial support by MCYT grant (AGL2001/2285) and predoctoral fellowship (to YT) is gratefully acknowledged.

REFERENCES

1. Klein Gebbinck E. A., Jansen B. J. M., de Groot A.: Phytochemistry *61*, 737 (2002).
2. Bruno M., Piozzi F., Rosselli S.: Nat. Prod. Rep. *19*, 357 (2002).
3. Ben Jannet H., Harzallah-Skhiri F., Mighri Z., Simmonds M. S. J., Blaney W. M.: Fitoterapia *71*, 105 (2000).

4. Piozzi F., Bruno M., Rosselli S.: Heterocycles *48*, 2185 (1998) and references cited therein.
5. Savona G., Passannanti S., Paternostro M. P., Piozzi F., Hanson J. R., Hitchcock P. B., Siverns M.: J. Chem. Soc., Perkin Trans. 1 *1978*, 356.
6. Savona G., Passannanti S., Paternostro M. P., Piozzi F., Hanson J. R., Siverns M.: Phytochemistry *17*, 320(1978).
7. Bruno M., Alcázar R., de la Torre M. C., Piozzi F., Rodríguez B., Savona G., Perales A., Arnold N. A.: Phytochemistry *31*, 3531 (1992).
8. Jakupovic J., Baruah R. N., Bohlmann F., Quack W.: Planta Med. *51*, 341 (1985).
9. Tonn C., Caltabiano J. A., Gianello J. C., Giordano O. S.: Rev. Latinoamer. Quim. *21*, 52 (1990).

BIOSYNTHESIS OF THE SESQUITERPENE LACTONE ESTER CNICIN IN *Cnicus benedictus* L.

DIRK UMLAUF*, HANS BECKER, KLAUS PETER ADAM*

Universität des Saarlandes, Pharmakognosie und Analytische Phytochemie, F.R. 8.7, Gebäude 32, 66041 Saarbrücken
E-mail: d.umlauf@mx.uni-saarland.de, k.adam@juno.com

Isoprenoids are the largest group of secondary metabolites. They share the same five carbon building block isoprene which is derived from isopentenyl diphosphate (IPP). This common precursor can be biosynthesized either *via* the mevalonic acid pathway (MVA) or *via* the methylerythritol phosphate pathway (MEP)[1,2]. Both MEP and MVA pathways are involved in the formation of isoprenoids of higher and lower plants.

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

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

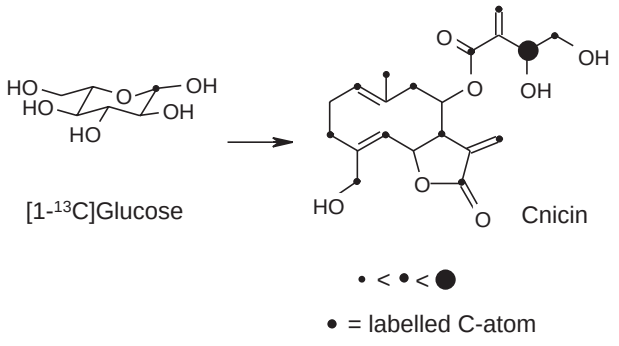


Fig. 1. Incorporation of [1-^{13}C]glucose and resulting labelling pattern of cnicin

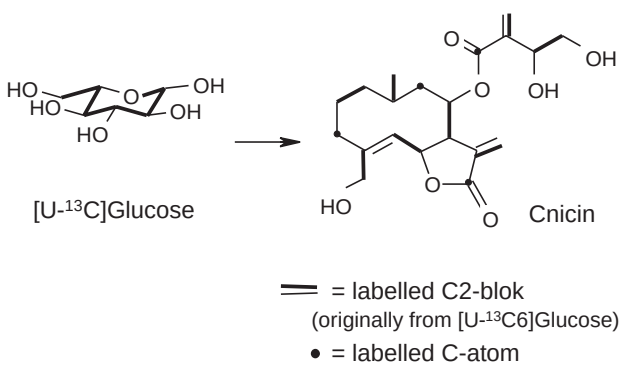


Fig. 2. Incorporation of [U-^{13}C]glucose and labelling pattern of cnicin

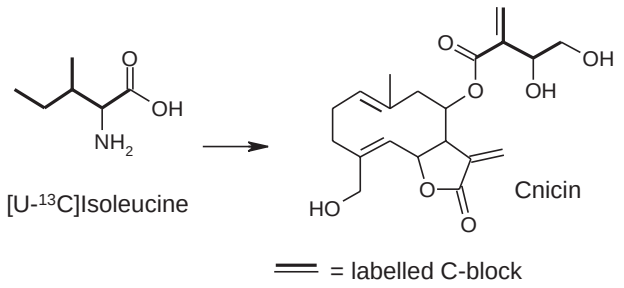


Fig. 3. Incorporation of [U-^{13}C]isoleucine and labelling pattern of cnicin

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

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

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

REFERENCES

1. Qureshi N., Porter J. W.: *Biosynthesis of Isoprenoid Compounds* (Porter J. W., Spurgeon S. L., ed.), Vol. 1, pp. 47–94. Wiley, New York 1981.
2. Rhomer M., Knani K., Simonin P., Sutter B., Sahm H.: Biochem. J. *295*, 517 (1993).
3. Adam K. P., Thiel R. Zapp J., Becker H.: Arch. Biochem. Biophys. *354*, 181 (1998).
4. Adam K. P., Zapp J.: Phytochemistry *48*, 953 (1998).
5. Dunstan R. H., Whatley F. R., Greenaway F. R. S., Greenaway W.: Proc. R. Soc. London, Ser. B *231*, 349 (1987).

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

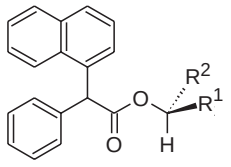
JAN VÁVRA[a], LUDVÍK STREINZ[b]*, MILOŠ BUDĚŠÍNSKÝ[b], BOHUMÍR KOUTEK[b]

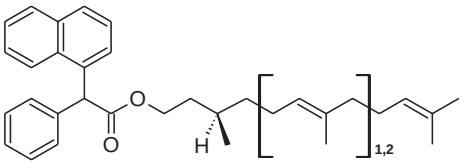
[a]Department of Organic Chemistry, Charles University, 120 00 Prague 2, [b]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic

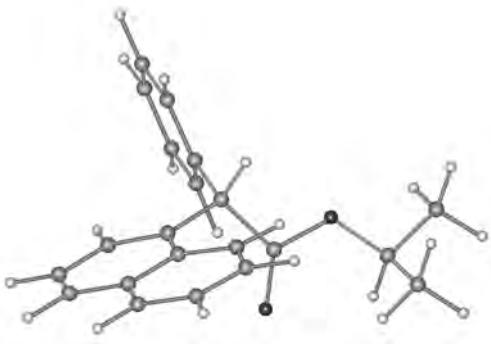
Mosher's acid (MTPA) has been one of the most favourite chiral derivatizing agents based on acetic acid pattern whose power lies in the anisotropic effect of an aromate. It has already proven its usefulness in the determination of the absolute configuration of chiral alcohols and amines. A new compound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

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

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







Supported by Research Project Z 4055 905 and the Grant Agency of the Czech Republic, grant No. 203/01/0116.

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

MARC VILAPLANA-POLO, ISMAEL ZAMORA, CARME BROSA*

Institut Químic de Sarrià, C.E.T.S., Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain
E-mail: brosa@iqs.url.es

In the field of brassinosteroids (BRs), we have developed a quantitative structure-activity relationship (QSAR) study that provide defined information about the minimum structural requirements necessary to elicit activity as plant growth promoters[1].

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

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

In our QSAR studies the "active conformation" of BRs was selected based on the methods of active analog approach (AAA) using geometrical descriptors as a selection criteria[1]. This approach consists on the selection of the "active conformation" for a reference compound (brassinolide) by comparing all its possible conformers with all the possible conformers of each of the other active BRs. Thus the "active conformation" of brassinolide will be that which results in highest

similarity. Once defined the "active conformation" for brassinolide, this is taken as reference for further comparison with all conformers of a defined BR. The "active conformation" of each BR will be the most similar to the "active conformation" of brassinolide.

According to these geometries very good QSAR models were obtained. Based on GRID methodology[2] we have found a good correlation between the activity and the areas with high probability of H-bonding with receptor[1]. The results obtained until now, suggested that the region near to 23*R*-OH was more important for eliciting activity than those near to 22*R*-OH.

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

REFERENCES

1. Brosa C.: *Structure-activity Relationship*, in: *Brassinostroids: Steroidal Plant Hormones* (Sakurai A., Yokota T., Clouse S., ed.) Chap. 9. Springer-Verlag, Tokyo 1999.
2. Wade R. C., Clark K. J., Goodford P. J.: J. Med. Chem. *36*, 140 (1993).
3. See poster Iban Jové et al.

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

EVA VLAŠÁNKOVÁ[a], LADISLAV KOHOUT[b], JAN HRADILÍK[a]

[a]Department of Botany and Plant Physiology, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemědělská 1, 613 00 Brno, [b]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: eva.vlasankova@seznam.cz

One of the most serious environmental stresses is drought. Not only survival during drought stress but also recovery of stressed plant is crucial for the next plant growth. Synthetic analogues of the brassinosteroids represent new plant growth regulators with the activity to increase plant growth but also to enhance resistence of plants to stress conditions. It was described[1] that 24-epibrassinolide increases root activity, plant growth and weight of the roots and shoots if applied as foliar spray to plants grown after water stress.

One of the first reactions of plants to stress is the ethylene production. The production of ethylene is increase by flooding, high temperature, toxic materials etc. Another phytohormone increase in water stress is abscisic acid in the leaves and roots[2].

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

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

This research was supported by the project of Czech Ministry of Education – FRVS 1190 and by projects Nos. 203/01/0083, A 4055204 and Z 4055905.

REFERENCES

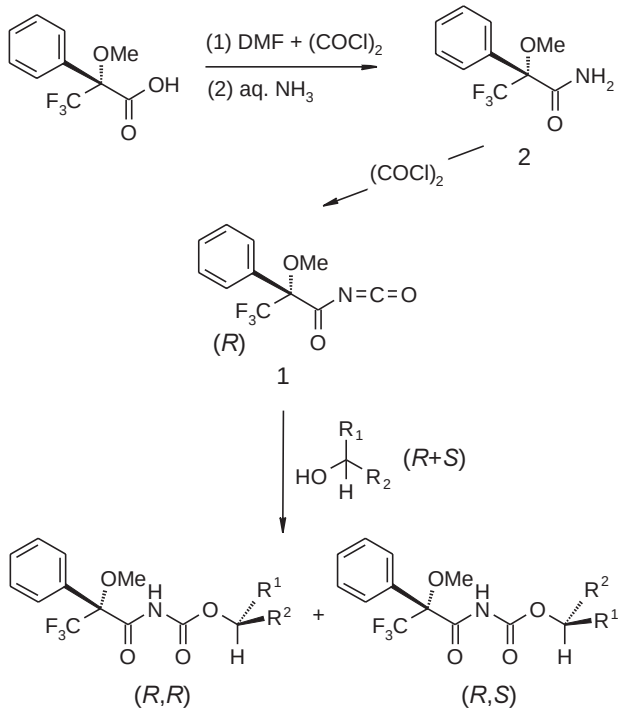
1. Khripach V. A., Zhabinskii V. N., de Groot A. E.: *Physiological Mode of Action of Brassinosteroids*, in: *Brassinosteroids: A New Class of Plant Hormones* (Khripach V. A., Zhabinskii V. N., de Groot A. E., ed.), pp. 220–301. Academic Press, San Diego 1999.
2. Macháčková I., in: *Regulátory rostlinného růstu* (Procházka S., Šebánek J., Macháčková I., Krekule J., Kamínek M., Borkovec V., Hradilík J., Havel L., Ondřej M., Psota V., Luxová M., Rauscherová L., Sladký Z., Vizárová G., Čížková R., Klíčová Š., Rozkošová V., ed.), pp. 96–99, 350–353. Academia, Prague 1997.

THE CHIRAL DERIVATIZATION WITH MOSHER'S ACID ACYLISOCYANATE

PETR VODIČKA, LUDVÍK STREINZ, MILOŠ BUDĚŠÍNSKÝ, BOHUMÍR KOUTEK

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: vodicka@uochb.cas.cz

Trichloroacetyl isocyanate (TAI) has alredy been proven as a valuable derivatization tool for NMR investigation[1]. Its chiral derivative, (*S*)-2-chloro-2-fluoroethanoyl isocyanate besides conserving good reactivity of TAI, brings additional informations with respect to the chirality of compound investigated, thus demonstrating the possible role of chiral acylisocyanates in the analysis of optically pure compounds[2]. For obtaining good results with those types of substances, however, the presence of some functional groups is essential. Namely, halogen atom(s) as well as aromatic ring in the molecule significantly accelerates the reaction and positively affects the spectral properties, respectively. Since the Mosher's acid[3] contains both the trifluoro- and phenyl groups, it seemed



Scheme1. Example for (*R*)-MTPA

to be a good starting material for preparing thus derived acylisocyanate.

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

Last but not least, results in the form of the characteristic chemical shifts have been compared with results of previous works where was used MTPA[3] and α-methoxy-α-(trifluoromethyl)benzyl isocyanate[4] as the chiral derivatization reagents.

Supported by Research Project Z 4055 905 and the Grant Agency of the Czech Republic, grant No. 203/01/0116.

REFERENCES

1. Goodlett V. W.: Anal. Chem. *37*, 431 (1965).
2. Vodička P., Streinz L., Koutek B., Buděšínský M., Ondráček J., Císařová I.: Chirality *15*, 472 (2003).
3. Dale J.A., Mosher H. S.: J. Am. Chem. Soc. *90*, 3732 (1968).
4. Nabeya A., Endo T.: J. Org. Chem. *53*, 3358 (1988).

SYNTHESIS OF STEROID CONTAINING MACROCYCLES *VIA* MULTI COMPONENT REACTIONS

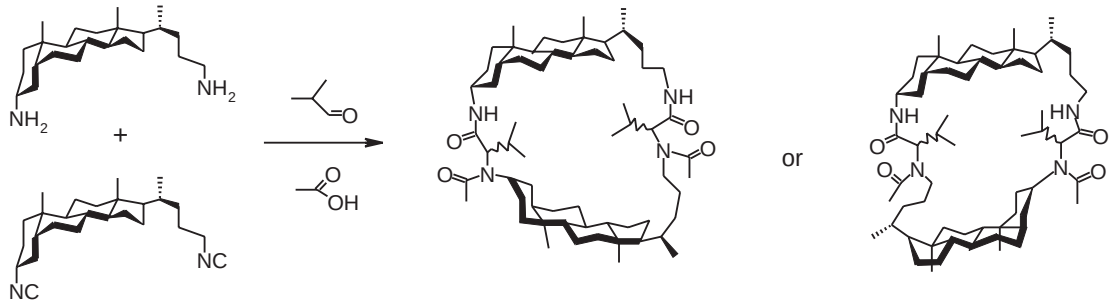
BRUNHILDE VOIGT, ANGELA SCHAKS, MONIKA BÖGEL, WOLFGANG BRANDT, LUDGER A. WESSJOHANN *

Institute of Plant Biochemistry, Weinberg 3, 06120 Halle/ Saale, Germany
E-mail: bvoigt@ipb-halle.de

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

With this background we started a program for the synthesis of macrocycles with steroid containing rigid parts using the Ugi-multi component reaction (U-MCR[1]). Bile acids[2] are the most valuable group of steroids due to their relatively rigid moiety, umbrella shape, chemically different hydroxyl groups, enantiomeric purity, availability, and low cost.

First step for the synthesis of cyclopeptide steroids is the bifunctionalization of bile acids as starting compounds. Good results were obtained with the U-4CR-condensation of the diamine[3] and diisocyanide synthesized from lithocholic acid, together with isobutyraldehyde and acetic acid (Scheme). After chromatography and preparative HPLC-separation eight



products were obtained having the molecular ion mass of 969 in the ESI-MS which showes the successful cyclization. These are the four possible diastereomers of the head-head and head-tail regioisomers. When paraformaldehyde was used for the same U-4CR only two main products were formed. Molecular modelling investigations showed that the heats of formation of the head to head and head to tail macrocycles is nearly the same, the probability of the formation of both macrocycles is close to equal. The cavities of synthesized macrocycles are large enough to encapsulate small organic substrates, and more detailed studies in this respect are under way.

REFERENCES

1. Dömling A., Ugi I.: Angew. Chem. *112*, 3300 (2000).
2. Tamminen J., Kolehmainen E.: Molecules *6*, 21 (2001).
3. Lawless L. J., Blackburn A. G., Ayling A. J., Perez-Payan M. N., Davis A. P.: J. Chem. Soc., Perkin Trans. 1 *2001*, 1329.

NONGENOMIC STEROID ACTION: FROM MEMBRANES TO HUMAN PHYSIOLOGY

M. WEHLING

Institute of Clinical Pharmacology, Faculty for Clinical Medicine Mannheim, University of Heidelberg, Germany

According to the traditional model steroid hormones bind to intracellular receptors and subsequently modulate transcription and protein synthesis, thus triggering genomic events finally responsible for delayed effects. Based upon similarities in molecular structure, specific receptors for steroids, vitamin D_3 derivatives, thyroid hormone, retinoids and a vanity of orphan receptors are considered to represent a superfamily of steroid receptors. In addition, very rapid effects of steroids mainly affecting intracellular signaling have been widely recognized which are clearly incompatible with the genomic model. These rapid, nongenomic steroid actions are likely to be transmitted *via* specific membrane receptors. Evidences for nongenomic steroid effects and distinct receptors involved are now presented for all steroid groups including related compounds like vitamin D_3 and thyroid hormones. The physiological and clinical relevance of these rapid effects is still largely unclear, but their existence *in vivo* has been clearly shown in various settings including human studies. Drugs that specifically affect nongenomic steroid action may find applications in various clinical areas such as cardiovascular and central nervous disorders, electrolyte homeostasis and infertility. In addition to a short description of genomic steroid action, this review pays particular attention to the current knowledge and important results on the mechanisms of nongenomic steroid action. The modes of action are discussed in relation to their potential physiological or pathophysiological relevance and with regard to a cross-talk between genomic and non-genomic responses.

Prominent examples of nongenomic steroid action are rapid aldosterone effects in lymphocytes and vascular smooth muscle cells, vitamin D_3 effects in epithelial cells, progesterone effects in human sperm, neurosteroid action on neuronal structures and vascular effects of estrogens. Mechanisms of action are being studied with regard to signal perception and transduction involved, and for various steroids including aldosterone a patchy sketch of a membrane receptor/second messenger cascade shows up in the mist being not essentially dissimilar to cascades involved in catecholamine or peptide hormone action. Aside nonclassical membrane receptors with a high affinity for a particular steroid, these effects appear to variably involve phospholipase C, phosphoinositide turnover, intracellular pH and calcium, protein kinase C and tyrosine kinases. The physiological and pathophysiological relevance of these effects is not yet clear, but more and more studies indicate that rapid steroid effects on cardiovascular, central nervous and reproductive functions occur *in vivo* and seemingly transmit physiological and pathophysiological responses.

Future research will have to target the cloning of the first membrane receptor for steroids which should be achieved in near future, and the evaluation of the physiological and clinical relevance of these rapid steroid effects.

REFERENCES

1. Wehling M., Christ M., Gerzer R.: Trends Pharmacol. Sci. *14*, 1 (1993).
2. Wehling M.: Annu. Rev. Physiol. *59*, 365 (1997).
3. http://steroid-rapid-responses.ucr.edu, 20/5 2003.

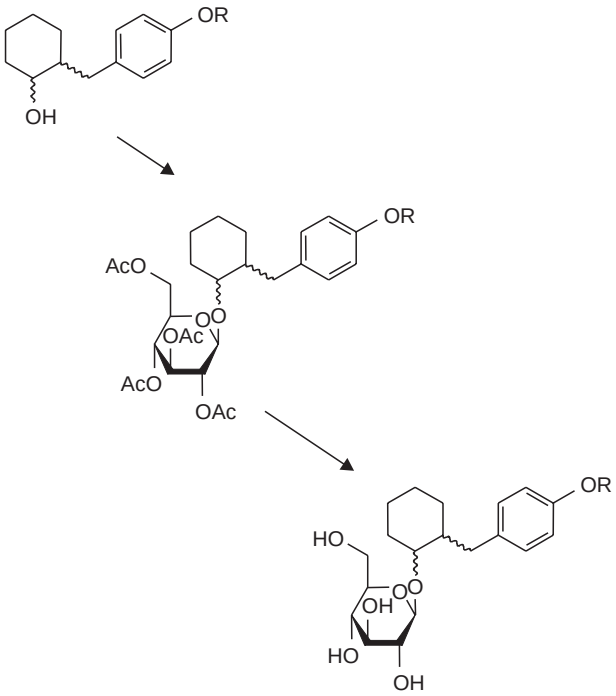
ALKYL GLYCOSIDES WITH BIOLOGICAL ACTIVITY

ZDENĚK WIMMER[a], PAVLA SOCHŮRKOVÁ[a], LAURA SĪLE[b], DAVID ŠAMAN[a], JELENA KULDOVÁ[a], IVAN HRDÝ[a]

[a]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic, [b]Latvian Institute of Organic Synthesis, Aizkraukles 21, 1006 Riga, Latvia

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

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



Scheme 1

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

This research was supported by the grant GA CR 203/02/0166. International collaboration was supported by the Czech-Latvian bilateral contract and has been a part of the COST D29/0002/03 network. The authors thank Mrs. M. Wimmerová for skillful technical assistance.

REFERENCES

1. Sláma K., Romaňuk M.: Insect Biochem. *6*, 579 (1976).
2. Wimmer Z., Rejzek M., Zarevúcka M., Kuldová J., Hrdý I., Němec V., Romaňuk M.: J. Chem. Ecol. *23*, 605 (1997).
3. Schmidt R. R., Jung K.-H., in: *Preparative Carbohydrate Chemistry* (Hanessian S., ed.), p. 283. Dekker 1997.

ROLES OF BRASSINOSTEROID BIOSYNTHESIS IN PEA SEED DEVELOPMENT AND GERMINATION

TAKAO YOKOTA, TAKAHITO NOMURA *

*Department of Biosciences, Teikyo University, Utsunomiya 320-8551, Japan (*Present address: Plant Science Center, RIKEN, Yokohama, 230-0045, Japan)*

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

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

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

In imbibed seeds, neither castasterone nor brassinolide were detected but the transcripts of *PsD*, *DDWF1*, *PsDWF4*, *PsBAS1* and *LKA* were increased. One and three days after the imbibition, castasterone was detected in germinating seeds. In these plantlets, the level of castasterone as well as the *PsD* expression was high in shoots and roots but low in seeds, indicating that the PsD protein seems to be a key enzyme for seed germination.

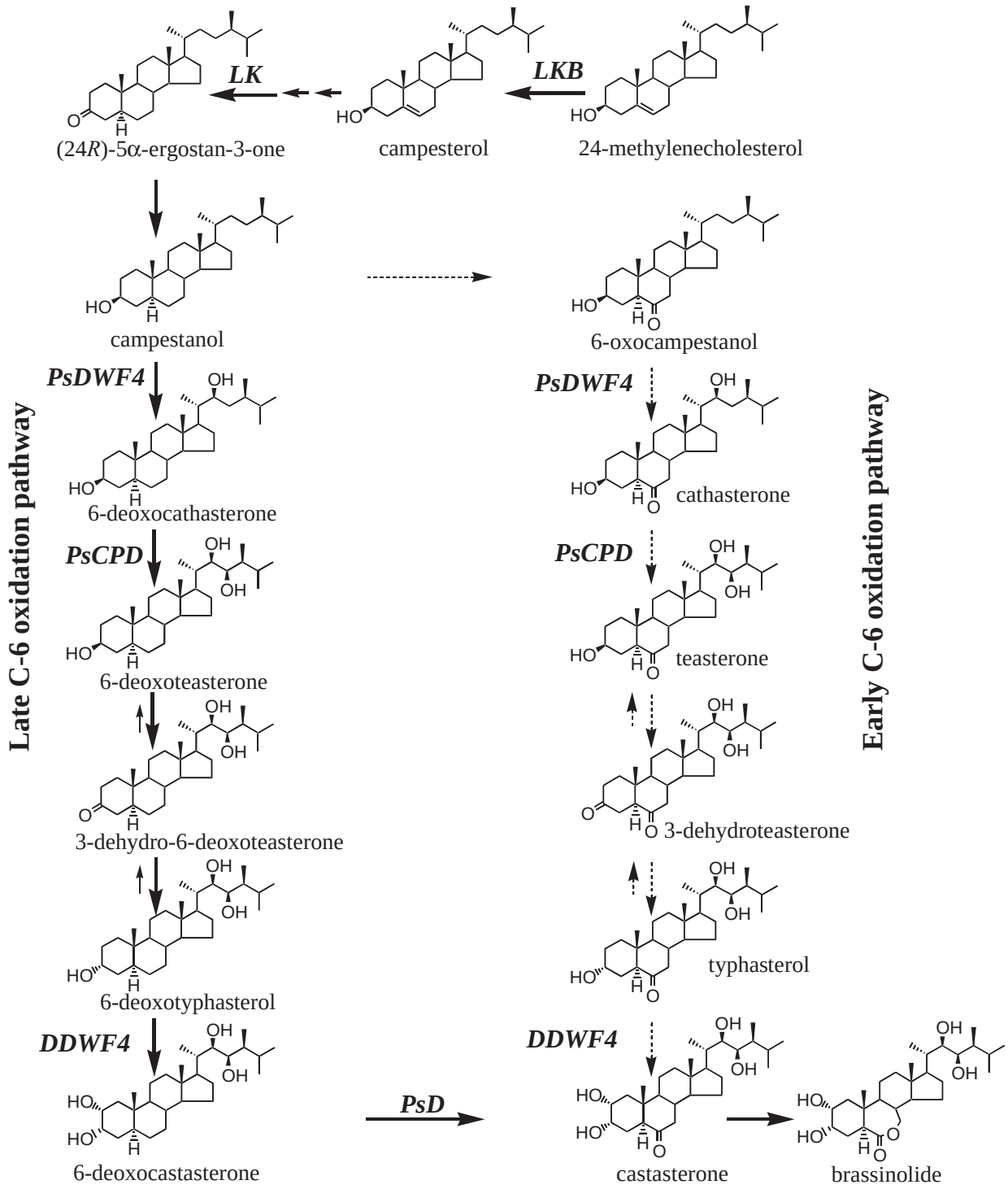


Fig. 1. Brassinosteroid biosynthesis and related genes in *Pisum sativum* (Bold arrows indicate major biosynthetic pathways)

SYNTHESIS OF 5,10- AND 13,14-SECOSTEROIDS

VLADIMIR N. ZHABINSKII[a], GALINA P. FANDO[a], ALLA A. GOVOROVA[a], MARINUS B. GROEN[b], AEDE DE GROOT[c], VLADIMIR A. KHRIPACH[a*], ANNA I. KOTYATKINA[a], JAAP VAN DER LOUW[b], ALEXANDER S. LYAKHOV[a], YULIYA Y. ZHIBURTOVICH[a]

[a]Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich Str., 5/2, 220141 Minsk, Belarus, [b]Department of Medicinal Chemistry, N.V. Organon, P.O. Box 20, 5340 BH Oss, [c]Wageningen University, Laboratory of Organic Chemistry, Dreijenplein 8, 6703 HB Wageningen, The Netherlands

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

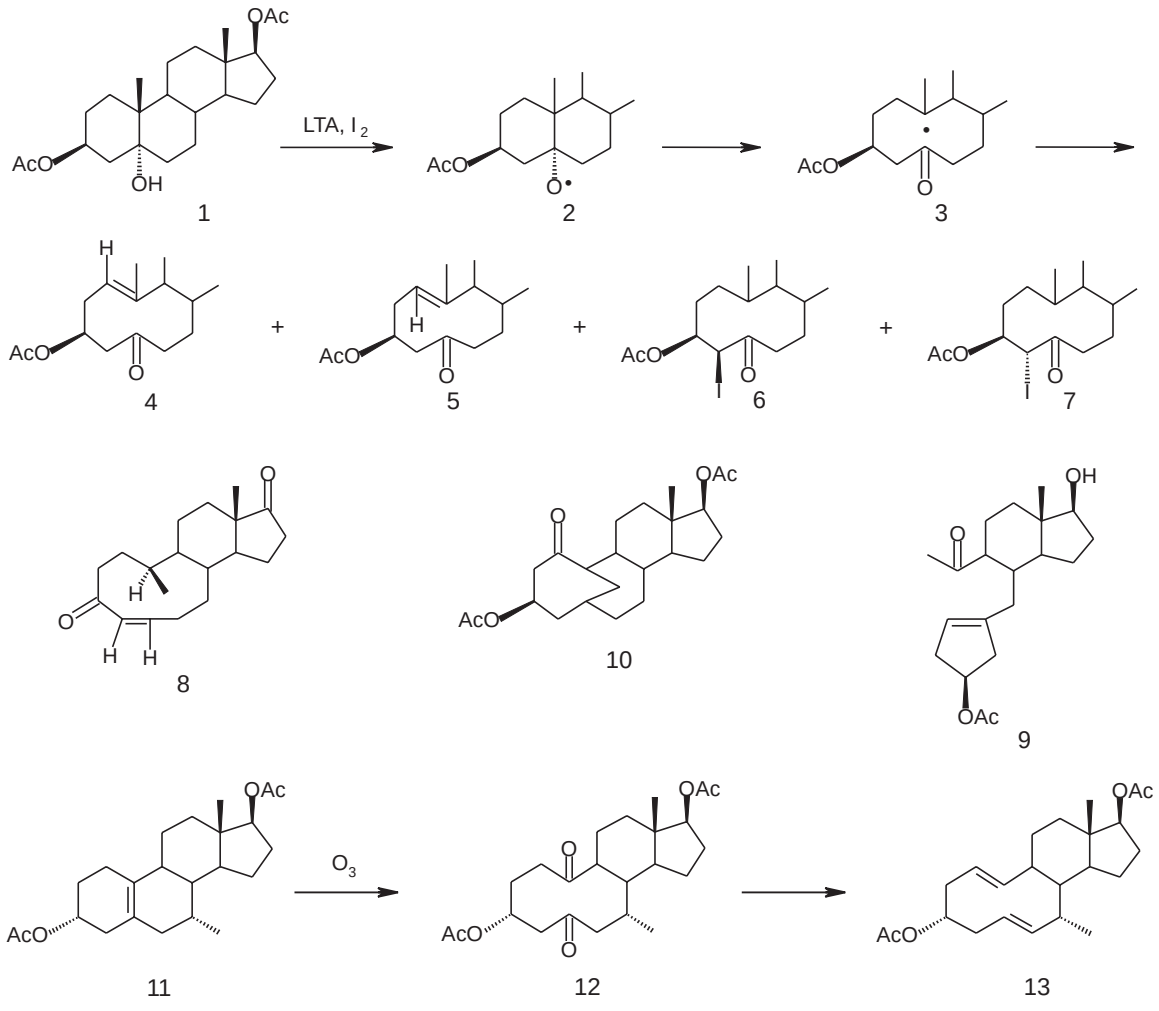
Radical oxidation of the tertiary alcohol 1 with lead tetraacetate in the presence of iodine gave a set of intermediates 4–7, which are suitable for the preparation of 5,10-seco steroids. The reaction proceeds *via* the radicals 2 and 3. The latter undergoes either hydrogen abstraction at C-1 to afford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

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

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

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

A second route to 13,14-seco steroids was based on the Grob fragmentation of hydroxy tosylate 20. Modifications of the resulting unsaturated ketone included hydride reduction of the 14-carbonyl group and hydroboration-oxidation of $\Delta^{13,17}$-



Author Index

CONFERENCE ON ISOPRENOIDS 2003
LATE PAPERS

These contributions were submitted so late after the deadline we were unable to include them into the indexed collection of abstracts. However, we did our best to let them appear despite the fact that the format, even after many editorial corrections, did not comply with the rules of the journal. We assume that sometimes the communication is more important than some formalities.

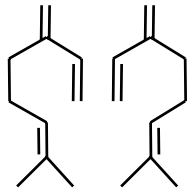
Editors

THE ORIGIN AND ACTIVITY OF ISOPRENOIDS IN PINES AND PINE BARK BEETLES

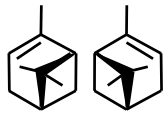
STEVEN J. SEYBOLD[1], MARTIN BLUMENBURG[2], JOCHEN TITZE[3], and WITTKO FRANCKE[3]

[1]Chemical Ecology of Forest Insects, USDA Forest Service, Pacific Southwest Research Station, 720 Olive Drive, Suite D, Davis, CA 95616 USA; [2]Institute of Biochemistry and Marine Chemistry, University of Hamburg, Bundesstrasse 55, 20146 Hamburg, Germany; [3]Institute for Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany
E-mail: sseybold@fs.fed.us

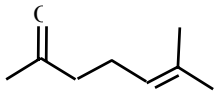
Pine-feeding bark beetles (Coleoptera: Scolytidae) interact with their host pines (Coniferales: Pinaceae) in the synthesis of one class of isoprenoids, the monoterpenoid aggregation pheromones. These pheromones are used to signal the mass attack of the beetles on pines, allowing the insects to coordinate feeding and mating in time and space. Examples of well-studied monoterpenoid pheromone components include ipsenol (**II**), ipsdienol (**III**), *cis*- and *trans*-verbenol (**V**, **VI**), and frontalin (**VIII**). Myrcene (**I**) is an acyclic pine oleoresin monoterpene that has been linked to the biosyntheses of ipsenol and ipsdieanol[1]; whereas α-pinene (**IV**) is a bicyclic pine oleoresin monoterpene that has been linked to the biosyntheses of *cis*- and *trans*-verbenol[2]. There is no obvious host monoterpene precursor for frontalin, but some have noted the presence of the geraniol derivative sulcatone (**VII**) in symbiotic fungi of bark beetles[3] and speculated that sulcatone may be an exogenous precursor to frontalin[4,5].



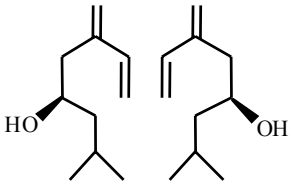
I. Myrcene



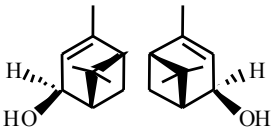
IV α-Pinene



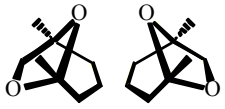
VII Sulcatone
6-methyl-5-hepten-2-one



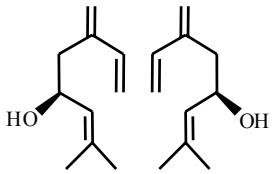
II Ipsenol
2-Methyl-6-methylene-7--octen-4-ol



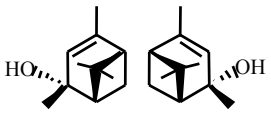
V *cis*-Verbenol
cis-4,6,6-trimethylbi-cyclo[3.1.1]hept-3-en-2-ol



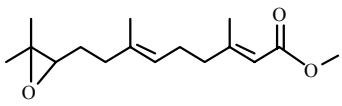
VIII Frontalin
1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane



III Ipsdienol
2-Methyl-6-methylene-
-2,7-octadien-4-ol



VI *trans*-Verbenol
trans-4,6,6-trimethylbi-
cyclo[3.1.1]hept-3-en-2-ol



IX Juvenile Hormone III
(±)-cis-10,11-epoxy-
3,7,11-trimethyl-E,E-2,6-
dodecadienoic acid
methyl ester

Fig. 1. Structures of isoprenoids in pines and pine bark beetles: pine monoterpenes, exogenous pheromone precursors, bark beetle aggregation pheromone components, and a bark beetle hormone.

A related structure, 6-methyl-6-hepten-2-one, has not been found in any hosts or symbionts of bark beetles, but may serve as the actual precursor to frontalin[6,7].

Research over the last 10 years has demonstrated that bark beetle pheromones can also be synthesized *de novo* (reviewed in[8]), with radiolabeling studies providing definitive proof for ipsenol and ipsdienol[9] and frontalin[10]. This endogenous synthesis occurs from acetate and mevalonate and is a highly regulated process that is stimulated at the transcriptional level by the sesquiterpenoid hormone, juvenile hormone III (JH III) (**IX**) (ref.[11-14]). In the case of *Ips* spp., the *de novo* biosynthesis of ipsenol and ipsdienol is evoked by feeding on the fresh phloem of the newly colonized host tree[11,15]; whereas in the case of the Jeffrey pine beetle, *Dendroctonus jeffreyi*, the *de novo* synthesis of frontalin appears to be initiated by emergence from the deteriorating phloem of the brood tree[16]. Whether *cis*- and *trans*-verbenol can be synthesized *de novo* by bark beetles remains an open question. There is some evidence from the mountain pine beetle, *Dendroctonus ponderosae*[17] and from *D. jeffreyi*[16] that the production of *trans*-verbenol by females can be stimulated by JH III.

Ultimately, the source of all carbon for pheromone production in bark beetles is from the food (i.e. pine phloem) ingested during the larval and adult stages[17]. However, we have only a rudimentary understanding of the degree of partitioning of bark beetle pheromone production between substrates originating from the host pine immediately prior to or during synthesis and substrates originating from the endogenous metabolic pool. To elucidate this biochemical insect-plant interaction requires focus on the late stages of the biosynthetic pathway (i.e. those reactions from isopentenyl diphosphate to the end products). Very recent work with the pine engraver, *Ips pini*, has demonstrated quite surprisingly that cell-free extracts of male tissue will convert geranyl diphosphate to myrcene[18]. This is the first evidence for a monoterpene synthase in the Metazoa and presents exciting new questions about the origin, evolution, and occurrence of terpene synthases in conifers and insects. This bark beetle monoterpene synthase is not only sex-specific, but its activity can be induced by prior treatment with JH III or by feeding on phloem from two host trees, Jeffrey pine, *Pinus jeffreyi*, and red pine, *Pinus resinosa*. The sex specificity and endocrine induction of this activity present a logical linkage of myrcene production in *I. pini* with pheromone biosynthesis. Ipsdienol is the principal pheromone component of *I. pini*[19], and this monoterpene alcohol can be synthesized from myrcene in this species[20].

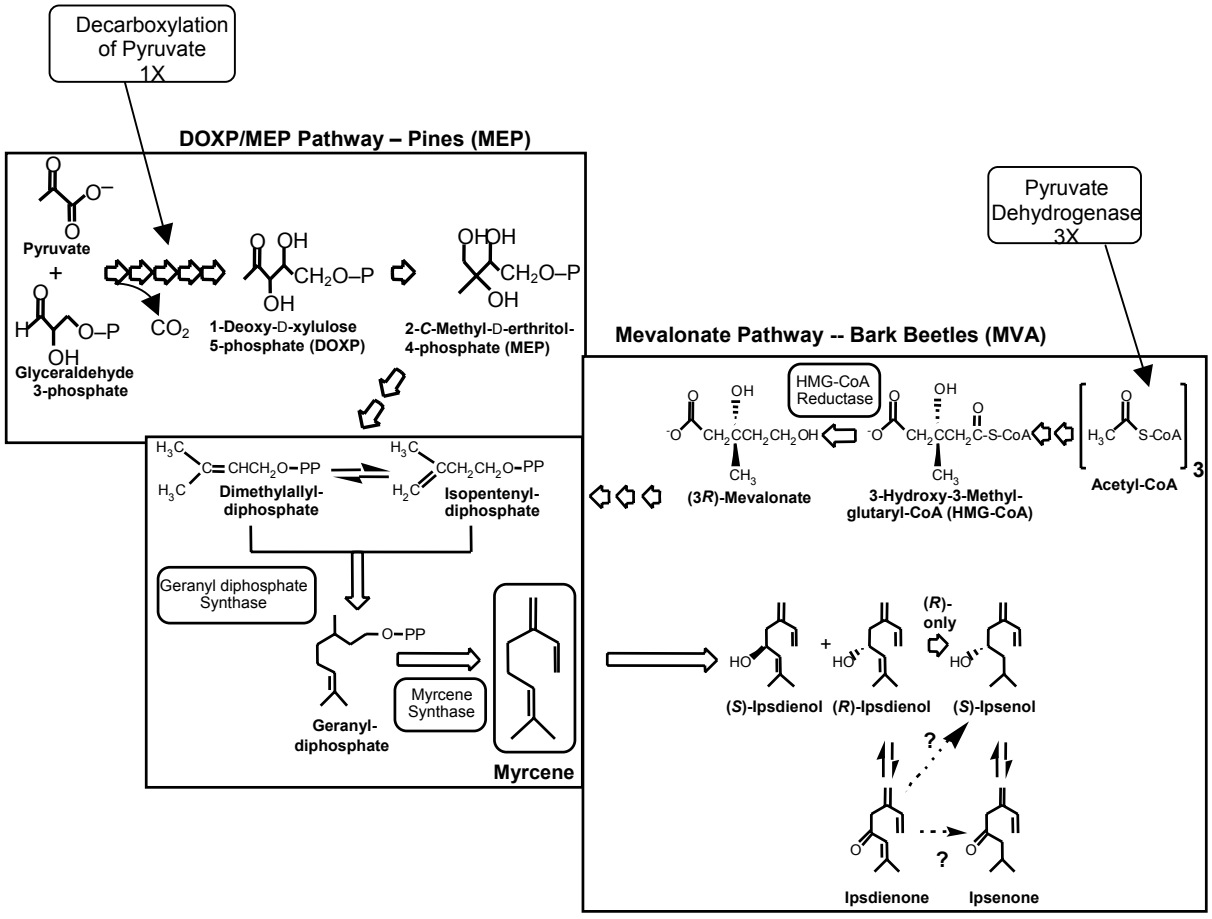
To further investigate the relationship between pine monoterpenes and bark beetle monoterpenoid pheromones, we have designed an experiment to compare the biosynthetic origins of myrcene and α-pinene from three species of pines with the biosynthetic origins of ipsenol, ipsdienol, *cis*- and *trans*-verbenol from three species of pine bark beetles. The pine engraver, *Ips pini*, the California fivespined ips, *I. paraconfusus*, and the pinyon ips, *I. confusus* (all bark beetles) were collected from Jeffrey pine, *Pinus jeffreyi*, ponderosa pine, *Pinus ponderosa*, and singleleaf pinyon pine, *Pinus monophylla*, respectively. These insects were collected from infested fallen stems and branches in the forests of the Sierra Nevada Mountains of California (*I. pini* and *I. paraconfusus*) or from infested standing trees in the forests of the Pinenut Mountains of Nevada (*I. confusus*). Concomitant with the collection of the insects, logs were collected from freshly cut live trees of the host pines. Newly emerged adult insects were reared from the infested pine logs, separated by sex, and stored temporarily at 4 °C on moist paper toweling.

Terpenoid-laden volatiles were trapped on Porapak Q (Supelco, Inc., Bellefonte, Pennsylvania, USA) by placing the freshly cut logs with and without artificially introduced *Ips* spp. into 19 l. glass carboys and drawing air for 7 to 10 days through the Porapak Q using published methods[19]. Samples were prepared with pheromone-producing males in the appropriate host logs (replicated 4 times), with non-pheromone producing females in the appropriate host logs (control, replicated once), and with only the host logs (control, replicated once). The 18 Porapak samples were extracted with pentane and the extracts will be analyzed by GC-FID, GC-MS, and isotope-ratio mass spectrometry (IRMS). Given comparative standard materials, this latter technique is a tool to determine the origin and history of organic compounds[21,22].

Hypothetically, the syntheses of monoterpenes by pines should occur in the plastids via the methylerythritol phosphate (MEP) pathway, whereas the monoterpene alcohols produced *de novo* by bark beetles should occur in the anterior midgut via the mevalonate (MVA) pathway. These two pathways, which

converge on isopentenyl diphosphate (Scheme 1), differ in the frequency with which the enzyme pyruvate dehydrogenase catalyzes the production of acetate from pyruvate. In the MEP pathway, the enzyme catalyzes one reaction as a prelude to the formation of 5-deoxy-D-xylulose-5-phosphate; whereas in the MVA pathway, mevalonate originates from three molecules of acetyl CoA (i.e. three catalytic events). As this thiamine-dependent pyruvate dehydrogenase prefers lighter isotopomers of pyruvate, i.e. those depleted in ^{13}C, a product of the MVA pathway should be relatively more depleted in ^{13}C isotopomers than a product of the MEP pathway. This difference in the endproducts of the MEP and MVA pathways can be measured using IRMS as the $\delta(^{13}C)$ value, which is expressed as $\delta(^{13}C)$ $[‰] = [(R_{sample}/R_{standard}) -1] \times 10^3$. R corresponds to the $^{13}C/^{12}C$ ratio of the sample and the standard (Vienna Pee Dee Belemite)[23]. The impact of the oxidation reactions that produce the monoterpene alcohols on the $\delta(^{13}C)$ value is not known.

The $\delta(^{13}C)$ values of monoterpenes have been reported in the literature to range from –26 to –30 ‰ (ref.[23]). Accordingly, preliminary results with the samples from *I. pini* show $\delta(^{13}C)$-values for myrcene of –27.1 ‰ (from volatiles collected from a log of the host tree, *P. jeffreyi*) and –27.5 ‰ (from volatiles collected from a log of *P. jeffreyi* containing male *I. pini*). The $\delta(^{13}C)$ value for ipsdienol from the same collection of volatiles above the headspace of feeding male *I. pini* was –25.1 ‰, which suggests that the oxidation reactions may dramatically increase the abundance of ^{13}C in the pheromone end product (even though it is synthesized via the MVA pathway). Results from the remainder of this study are pending analysis.



Scheme 1. Proposed interaction of monoterpenoid biosynthetic pathways in pines and bark beetles showing hypothetical alternatives for incorporation of ^{13}C resulting from decarboxylation of pyruvate in the MEP and MVA pathways[18].

METHODS

Coupled gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) was conducted using a Finnigan MAT 252 mass spectrometer equipped with a ThermoFinnigan Trace GC gas chromatograph and CuO/Ni/Pt combustion furnace operated at 940 °C. The samples were injected splitless (0.8 min) onto a 30 m fused silica column (DB5-MS, 0.32 mm i.d., 0.25 µm film thickness). Injector temperature was 220 °C. Carrier gas: He. GC temperature program: 3 min 45 °C; 45 °C to 220 °C at 3 °C min^{-1} ; 10 min

220 °C. Standard deviations for replicate injection (3) varied from 0.1 to 0.5 ‰ and were typically less than 0.2 ‰. The stable carbon isotope compositions are reported in the δ notation against the VPDB ^{13}C-Standard.

REFERENCES

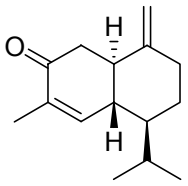
1. Hendry L.B., Piatek B., Browne L.E., Wood D.L., Byers J.A., Fish R.H., Hicks R.A.: Nature *284*, 485 (1980).
2. Renwick J.A.A., Hughes P.R., Krull I.S.: Science *191*, 199 (1976).
3. Brand J.M., Barras S.J.: Lloydia *40*, 398 (1977).
4. Brand J.M., Young J.C., Silverstein R.M.: Prog. Org. Chem. Nat. Prod. *37*, 1 (1979).
5. Vanderwel D., Oehlschlager A.C.: *In Pheromone Biochemistry*, (ed. G.D. Prestwich, G.J. Blomquist), pp. 175-215, Orlando, Academic Press (1987).
6. Francke W., Bartels J., Meyer H., Schröder F., Kohnle U., Baader E., Vité J.P.: J. Chem. Ecol. *21*, 1043 (1995).
7. Perez A.L., Gries R., Gries G., Oehschlager A.C.: Bioorg. Med. Chem. *4*, 445 (1996).
8. Seybold S.J., Tittiger C.: Ann. Rev. Entomol. *48*, 425 (2003).
9. Seybold S.J., Quilici D.R., Tillman J.A., Vanderwel D., Wood D.L., Blomquist G.J.: Proc. Natl. Acad. Sci. USA 92, 8393 (1995b).
10. Barkawi L.S., Francke W., Blomquist G.J., Seybold S.J.: Insect Biochem. Mol. Biol. (In Press) (2003).
11. Tillman J.A., Holbrook G.L., Dallara P.L., Schal C., Wood D.L., Blomquist G.J., Seybold S.J.: Insect Biochem. Mol. Biol. 28, 705 (1998).
12. Tillman J.A., Lu F., Donaldson Z., Dwinell S.C., Tittiger C., Hall G.M., Blomquist G.J., Seybold, S.J.: *Meeting Abstract, Annu. Meet. Int. Soc. Chem. Ecol., 18th*, Lake Tahoe, California (2001).
13. Tittiger C., Blomquist G.J., Ivarsson P., Borgeson C.E., Seybold S.J.: Cell. Mol. Life Sci. *54*, 121 (1999).
14. Tittiger C., Barkawi L.S., Bengoa C.S., Blomquist G.J., Seybold S.J.: Mol. Cell. Endocrinol. *199*, 11 (2003).
15. Wood D.L.: Pan-Pacific Entomol. *38*, 141 (1962).
16. Barkawi L.S.: Ph.D. Dissertation, University of Nevada, Reno, 193 pp. (2002).
17. Conn J.E., Borden J.H., Hunt D.W.A., Holman J., Whitney H.S., Spanier O.J., Pierce H.D., Jr., Oehschlager A.C.: J. Chem. Ecol. *10*, 281 (1984).
17. Seybold S.J., Bohlmann J., Raffa K.F.: Can. Entomol. *132*, 697 (2000).
18. Martin D., Bohlmann J., Gershenzon J., Francke W., Seybold S.J.: Naturwissenschaften *90*, 173 (2003).
19. Seybold S.J., Ohtsuka T., Wood D.L., Kubo I.: J. Chem. Ecol. *21*, 995 (1995a).
20. Vanderwel D.: Ph.D. Dissertation, Simon Fraser University, 172 pp. (1991).
21. DeNiro M.J., Epstein S.: Science *197*, 261 (1977).
22. Teece M.A., Fogel M.L., Dollhopf M.E., Nealson K.H.: Org. Geochemistry *30*, 1571 (1999).
23. Jux A., Gleixner G., Boland W.: Angew. Chem. Int. Ed. *40*, 2091 (2001).

FUNGAL TRANSFORMATION OF SOME TERPENES AND STEROIDS

PAUL B. REESE
Department of Chemistry, University of the West Indies, Mona Campus, Kingston 7, Jamaica

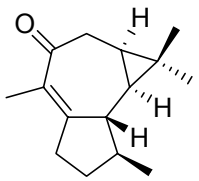
Jamaica is geographically situated in the centre of the Caribbean. The island boasts a number of micro-climates and this is reflected in the wide diversity of plant and microbial life. There are over 3,000 recorded flowering plants; a quarter of which are endemic[1]. Phytochemical analysis of some members of three plant families has been performed. Some of the biologically active terpenes isolated have been incubated with fungi in the hope of generating a series of analogues. Furthermore, for the first time, two locally isolated fungi have been examined for their potential for steroid transformation.

Hyptis verticillata, an example from the Labiatae, is used in traditional medicine to treat bronchial disorders. A number of terpenes (e.g. **I**, **II**)[2,3], flavonoids and lignans[4-6] have been isolated from this aromatic herb. Compound I has been shown to reduce the fertility of the cattle tick, *Boophilus microplus*, while both **I** and **II** are toxic to the sweet potato weevil, *Cylas formicarius elegantulus*[2,3].



I

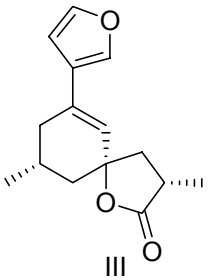
I – cadinane



II

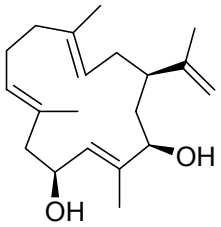
II - aromadendrane

Capraria biflora, a member of the Scrophulariaceae family, has been used in local folklore for various bacterial and viral infections. Although the extracts contain a large number of compounds, careful chromatographic separation has yielded four sesquiterpenes (e.g. **III**). Such caprariolides, as they have been called, possess a novel skeleton and have been found to exhibit insecticidal activity[7].



III

III - caprariolide A

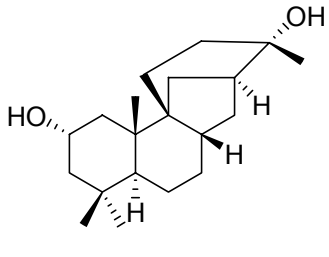


IV

IV - cembrane derivative

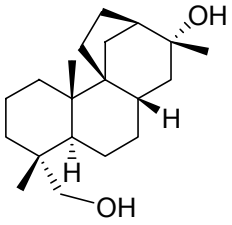
Cleome spinosa, from the Capparaceae, yields large amounts of a new cembrane (**IV**) along with four congeners[8]. Terpenes with the cembrane skeleton are known to possess

diverse biological activity including potent cytotoxicity against a number of human cancer cell lines, inclusive of leukaemia, melanoma, breast and colon carcinomas.



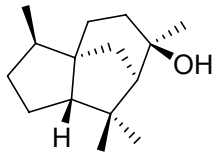
V

V - stemodin



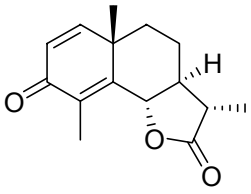
VI

VI - stemarin



VII

VII - cedrol

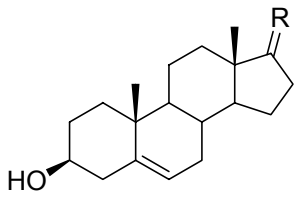


VIII

VIII - santonin

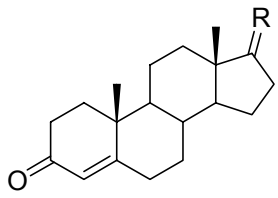
The incubation of some of these terpenes and others (e.g. **V-VIII**) as well as their analogues with the fungi *Beauveria bassiana*, *Rhizopus arrhizus*, *Mucor plumbeus*, *Curvularia lunata* and *Aspergillus niger* has been effected.

This has led to the preparation of a wide range of compounds, many which are products of hydroxylation. Some of these metabolites are novel and a number possess enhanced biological activity[9-14].



IX: R=O
X: R=βCH$_3$CO, αH

IX - DHEA
X - pregnenolone



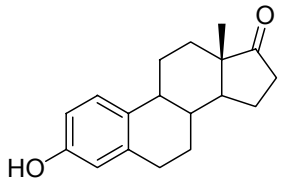
XI: R=βOH, αH
XII: R=βCH$_3$CO, αH

XI - testosterone
XII - progesterone

The fungus *Fusarium oxysporum* var. *cubense* is the causative agent of the dread Panama disease of bananas (*Musa* sp.).

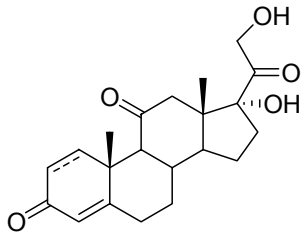
This deuteromycete grows well in liquid culture and has been found to bring about 7α hydroxylation on steroids **IX** and **X**. Substrates **XI-XIII** were functionalised in the C-15α position. **XIV** and **XV** underwent side chain cleavage.

Some redox reactions also accompanied these transformations[15].



XIII

XIII - estrone



XIV: Δ^4
XV: $\Delta^{1,4}$

XIV - cortisone
XV - prednisone

Exophiala jeanselmei var. *lecanii-corni* was encountered as a contaminant of a ginger plant (*Zingiber officinale*). The fungus, which belongs to the Ascomycotina, effected side chain degradation on **X**, **XIV** and **XV**; 1,2- and 1,4 reduction of enones **XI**, **XIV** and **XV**; and redox chemistry on most substrates. Epoxidation, followed by rearrangement, was observed when **IX** was incubated with the fungus[16].

The feasibility of terpene transformation, by these fungi, will be investigated in the future.

REFERENCES

1. Adams C.D.: *Flowering plants of Jamaica*. MacLehose & Co., Glasgow, 1972.
2. Porter R.B.R., Reese P.B., Williams L.A.D., Williams D.J.: Phytochemistry *40*, 735 (1995).
3. Collins D.O., Buchanan G.O., Reynolds W.F., Reese P.B.: Phytochemistry *57*, 377 (2001).
4. German V.F.: J. Pharm. Sci. *60*, 649 (1971).
5. Kuhnt M., Rimpler H., Heinrich M.: Phytochemistry *36*, 485 (1994).
6. Novelo M., Cruz J.G., Hernandez L., Pereda-Miranda R.: J. Nat. Prod. *56*, 1728 (1993).
7. Collins D.O., Gallimore W.A., Reynolds W.F., Williams L.A.D., Reese P.B.: J. Nat. Prod. *63*, 1515 (2000).
8. Collins D.O., Reynolds W.F., Reese, P.B., unpublished material.
9. Collins D.O., Reese P.B.: Phytochemistry *56*, 417 (2001).
10. Collins D.O., Buchanan G.O., Reynolds W.F., Reese P.B.: Phytochemistry *57*, 377 (2001).
11. Chen A.R.M., Reese P.B.: Phytochemistry *59*, 57 (2002).
12. Collins D.O., Ruddock P.L., Chiverton de Grasse J., Reynolds W.F., Reese P.B.: Phytochemistry *59*, 479 (2002).
13. Collins D.O., Reese P.B.: Phytochemistry *59*, 489 (2002).
14. Collins D.O., Reynolds W.F., Reese P.B.: Phytochemistry *60*, 475 (2002).
15. Wilson M.R., Gallimore W.A., Reese P.B.: Steroids *64*, 834 (1999).
16. Porter R.B.R., Gallimore W.A., Reese P.B.: Steroids *64*, 770 (1999).

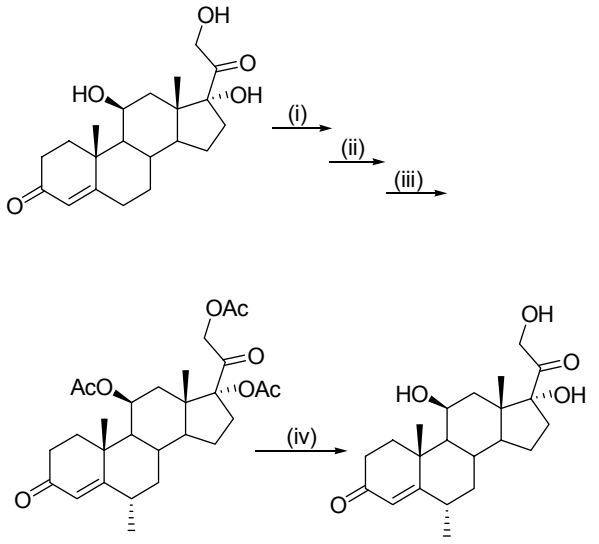
A NEW EFFECTIVE APPROACH TO 6α-METHYLHYDROCORTISONE

NIKOLAI V. LUKASHEV, GALINA S. GRINENKO*, ALEXEI V. TSVETKOV, ALEXEI V. KAZANTSEV and GEORGE A. SKRYABIN**

*The Department of Chemistry, Moscow State Lomonosov University, Vorobievy Gory, 119992, Moscow, Russia, *Centre for Drug Chemistry, Zubovskaya Str., 7, Moscow 119815, Russia; **Ambiotech, Inc., USA.*
E-mail: lukashev@aha.ru

6α-methylprednisolone (medrol, methypred, etc.) is among very important pharmaceuticals with anti-inflammatory, antiallergenic and immunosuppressant effect, which is by 6-7 times as active as its nearest analogue – prednisolone. Moreover 6α-methylprednisolone does not posses mineral corticoid (sodium-arresting) after-effect. A propitious approach to 6α-methylprednisolone is a microbiological 1,2-dehydrogenation of 6α-methylhydrocortisone[1,2]. Although the latter compound is of vital importance, there are very few publications which deal with its chemistry and synthesis[3].

We have reproduced these approaches and discovered that they all are extremely laborious and time-consuming, requiring meticulous protection/deprotection procedures to ensure tolerance to the existing functionality thus leading to essential losses and decrease of net yield. As the synthesis of hydrocortisone from naturally occurring sterols (*via* androst-4-en-3,17-dione and then cortexolone 21-acetate) is a well-known and optimised procedure[2], we decided that an effective approach to 6α-methylhydrocortisone synthesis should proceed through a triester of hydrocortisone.



(i) Ac_2O, pyridine, CH_2Cl_2; (ii) $(MeO)_2CH_2$, $POCl_3$, NaOAc, $CHCl_3$; (iii) Pd/C, cyclohexene, EtOH, (iv) *Corynebacterium mediolanum ABT-AL-301*

We have found that hydrocortisone can be easily transformed to its 11,17,21-triacetate in 98% yield. Introduction of 6-methyl group can be achieved via methylenation[4] or through Vilsmeier formylation[5] with subsequent hydrogenation in 70% total yield. It is known that subsequent hydrolysis of all ester groups is complicated by an easy elimination of hydroxyacetyl group in 17-position. We have shown that more than 97% yield of 6α-methylhydrocortisone can be achieved during hydrolysis of starting triester by means of *Corynebacterium mediolanum ABT-AL-301*.

The data obtained allow us to improve industrial synthesis of 6α-methylprednisolone from sterols in more cost effective way.

REFERENCES

1. A. Arinbasarova et al., Enz. & Microb. Techn. *19*, 337 (1996).
2. Ambiotech, Inc., Review 2002.
3. Pat. USA 3141876, Pat. FRG 1618 599, & some other patents of Upjohn Comp. or Schering AG.
4. K.Annen et al., Synthesis, *1982*, 34.
5. D. Burn et al., Tetrahedron *20*, 597 (1964).

TERPENE FORMATION IN MAIZE AND ITS ECOLOGICAL AND EVOLUTIONARY SIGNIFICANCE

TOBIAS G. KÖLLNER, CHRISTIANE SCHNEE, JONATHAN GERSHENZON, and JÖRG DEGENHARDT

Max Planck Institute for Chemical Ecology, Beutenberg Campus Winzerlaer Strasse 10, D-07745 Jena, Germany
E-mail: degenhardt@ice.mpg.de

Despite the remarkable abundance and diversity of terpenoid secondary metabolites in plants, there are still large gaps in our knowledge of their biological functions and evolutionary origin. However, the availability of genetic and genomic resources for certain model plant species provides an exciting array of new tools for exploring the ecological and evolutionary significance of this enormous class of natural products. We have begun to employ genetic and genomic tools to study terpene biosynthesis in corn (*Zea mays)*, focusing on the genes of the terpene synthase family which encode the major group of enzymes controlling the formation of terpenoid secondary metabolites. By carrying out sequence comparisons, functional characterization and gene expression studies along with profiling terpenoid metabolites, we have gained new information about the physiology, ecology and evolution of monoterpenes and sesquiterpenes in this species.

The C_{10} and C_{15} terpenoids of maize are not associated with specialized oil cells, ducts, trichomes or other secretory cavities and are present at low levels throughout the plant. Damage to the plant by lepidopteran larvae, such as the beet army worm (*Spodoptera exigua*) and the European corn borer (*Ostrinia nubilalis*), results in the release of volatiles, including terpenoids, indole, and products of the lipoxygenase pathway

into the headspace of the plant. This volatile blend attracts herbivore enemies like the parasitoid *Cotesia marginiventris*, a braconid wasp (Turlings et al., 1990, 1991). *Cotesia marginiventris* females lay their eggs on *S. exigua* caterpillars and the parasitoid larvae that emerge begin to consume their insect host leading to its eventual death. Since caterpillars parasitized by *C. marginiventris* consume significantly less plant tissue than unparasitized caterpillars (Turlings and Fritzsche, 1999), such tritrophic interactions can be of significant benefit to the plant and are termed indirect defense. In addition, the volatility and reactivity of these substances support a protective function against oxidative damage, analogous to that proposed for isoprene.

The universal occurrence of monoterpenes and sesquiterpenes in higher plants also argues that these substances appeared early in angiosperm evolution. If so, how can one account for the bewildering differences in terpene composition within and among plants? It has previously been established that within plant diversity can often be attributed to the ability of individual terpene synthase enzymes to make multiple products. Indeed, we have found that multi-product terpene synthases appear to be just as prevalent in maize as in classical terpene-accumulating species, such as labiates and conifers. To explain the origin of terpene diversity among grasses, we have employed terpene synthase sequence comparison, mapping experiments and genomic sequence

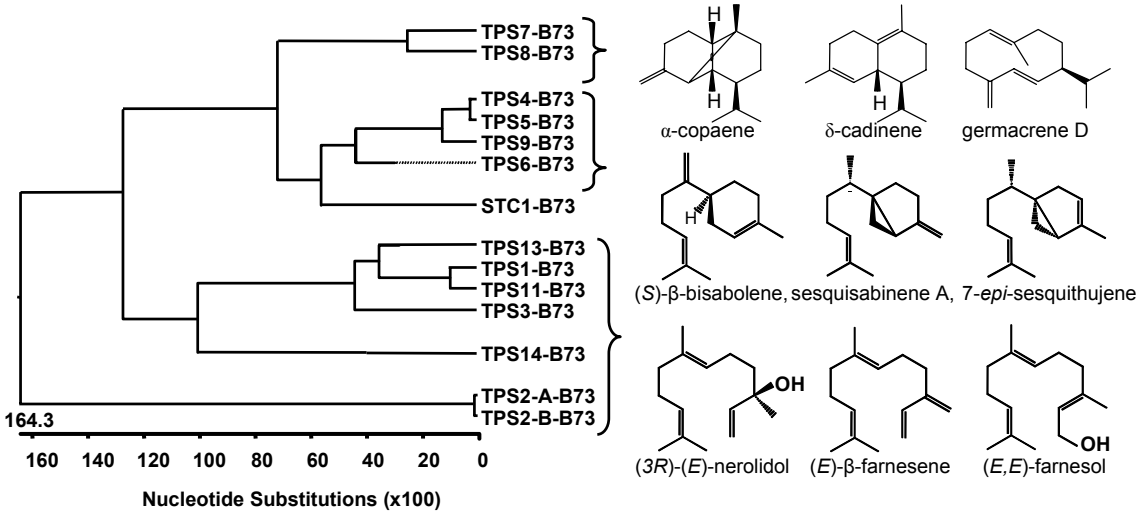


Figure 1: Dendrogram analysis of the maize terpene synthases based on amino acid sequence identity. Examples of the major products of the terpene synthases within each subgroup are given.

information to search for the signatures of past evolutionary processes. Dendrogram analysis of the maize terpene synthases based on amino acid sequence identity revealed a very diverse group of enzymes that forms several subgroups (Figure 1). The enzymes TPS1, TPS2 and TPS11 form acyclic olefins and terpene alcohols from the substrate farnesyl diphosphate. These enzymes share a fairly simple reaction mechanism, but do not necessarily have a high sequence identity which suggests an instance of convergent evolution. The enzymes TPS7 and TPS8 have related amino acid sequences and form mostly bicyclic sesquiterpenes of the cadinane type with a partially overlapping product spectrum. Many of the monocyclic terpenes of maize are formed by the enzyme cluster TPS4, TPS5 and TPS10.

To reconstruct scenarios involving gene duplication and subsequent divergence, we studied the terpene synthases TPS4 and TPS5 in the maize varieties B73 and Delprim. The two enzymes are encoded by separate genes on chromosome 10 and share 96% identity on the amino acid level. Both convert farnesyl diphosphate to a complex blend of approximately 20 sesquiterpenes dominated by sesquithujane-, sesquisabinane-, bergamotane- and bisabolane-type olefins, but the two enzymes favor the formation of distinct stereoisomers resulting in a substantial difference in their product spectra (Figure 2). Site directed mutagenesis revealed that only four amino acid residues in the catalytic center control its stereoselectivity, with the most dramatic change in product profile being observed upon the substitution of an alanine by a glycine. Structural models of the catalytic center suggest that minor changes in the wall of the cavity are causing the stereoselectivity of the enzymes.

To determine why emissions of mature B73 plants are dominated by TPS4 products while those of mature Delprim plants are dominated by TPS5 products, we searched both varieties for alleles of tps4 and tps5. In B73, an active tps4 allele is present, but the protein encoded by the *tps5* allele is catalytically inactive when expressed heterologously.

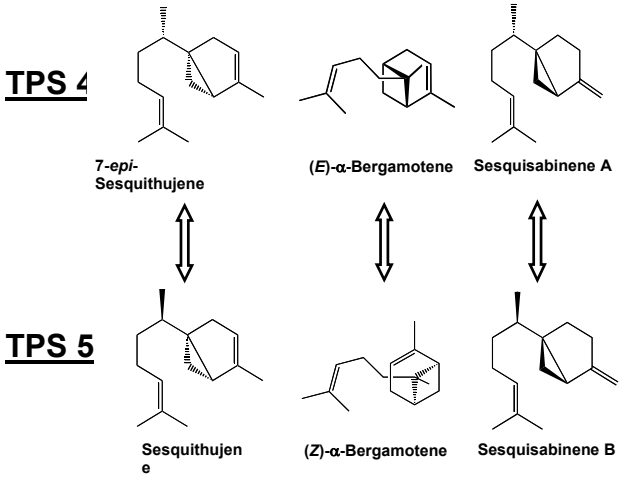


Figure 2: The terpene synthases TPS4 and TPS5 form a very similar group of sesquiterpene olefins with many of the products being stereoisomers of each other.

Site-directed mutagenesis of this inactive allele showed that alteration of two amino acid residues was all that was necessary to render it functional. Conversely, the cultivar Delprim harbors an active *tps5* allele, but its *tps4* alleles are non-functional due to a frameshift mutation. These results suggest that substantial differences in terpene profiles can be controlled by alleles possessing only minor sequence differences.

It is most likely that *tps4* and *tps5* are the result of a recent gene duplication and diversification within the last five million years. Many studies have observed that after gene duplication, one of the two duplicated genes loses its activity due to the functional redundancy of the encoded proteins. This loss of activity might proceed via the gradual accumulation of non-functional alleles within the plant population. It is conceivable that this process is responsible for the surprisingly high number of inactive *tps4* and *tps5* alleles. Since terpene synthases are probably not be essential for plant survival under many growing conditions, the accumulation of mutations might be somewhat higher than in genes of primary metabolism. The higher mutation rate affecting the product specificity of the terpene synthase might be advantageous to the plant to acquire new defenses against large numbers of constantly adapting enemies. The strong expression of the terpene blends in leaves and husks in plants after anthesis suggests a role as toxin or feeding deterrent. Further studies will be necessary to evaluate the contribution of sesquiterpenes to the defense against herbivores or fungal pathogens of maize.

LIGAND RECOGNITION BY PROGESTERONE RECEPTORS FROM FILAMENTOUS FUNGUS *RHIZOPUS NIGRICANS* EXTENDS TO ARYLHYDROCARBONS AND FLAVONOIDS

HELENA LENASI

Medical Faculty, Institute of Biochemistry, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia
E-mail: helena.lenasi@mf.uni-lj.si

A saprophytic fungus *Rhizopus nigricans* (*R. nigricans*) can thrive in several natural and artificial conditions since it contains an effective adaptation system. An important part of its capability to acclimatize to several environmental compounds represents the detoxification system containing cytochrome P450 which transforms different fungitoxins into less toxic products[1]. In this way mammalian hormone progesterone is rendered into harmless 11α-hydroxyprogesterone[2]. Progesterone-hydroxylase is inducible by the substrate progesterone[3] and it seems that progesterone receptors are involved in this progesterone singnaling[4]. In the presented study we characterized progesterone receptors in *R. nigricans* cytosol with respect to steroidal and nonsteroidal ligand specificity. In addition, we examined the effect of selected ligands on hydroxylase induction by progesterone.

In competition studies 40 nM (^{3}H)-progesterone was used. Out from steroids 3,20-keto-pregnanes were the best ligands defined by EC_{50} of 2.2±0.5 x 10^{-7} M. Reduction of C3-oxo and elimination of C17 side-chain significantly decreased the affinity for receptors. Among nonsteroidal arylhydrocarbons, known as environmental pollutants, α-naphthoflavone (EC_{50}=3.2±0.4 x 10^{-8} M), β-naphthoflavone and benzo(a)pyrene competed effectively with progesterone for progesterone receptors, but β-naphthoflavone and benzo(a)pyrene were not

able to displace labeled progesterone to the same level as nonlabeled progesterone. Most likely, these compounds are bound to an allosteric site. Moreover, some natural flavonoids were examined as progesterone receptor ligands. The obtained competition curves did not reach the bottom level of nonlabeled progesterone; their apparent EC_{50} values were as follows: flavone, 1.9±0.8 x 10^{-6} M; kaempferol, 1.7±0.6 x 10^{-6} M; apigenin, 8.1±0.9 x 10^{-6} M; isoflavone genistein, 1.1±0.8 x 10^{-5} M.

Progesterone receptor ligands were used as inducers of progesterone hydroxylase. A clear dose-dependent enzyme induction was obtained with high affinity ligands (progesterone, deoxycorticosterone and testosterone), whereas no induction was observed with low affinity ligand estradiol (Fig. 1). Furthermore, nonsteroidal good progesterone competitors were examined for their ability to interfere with hydroxylase induction by progesterone. α-naphthoflavone inhibited the induction in a dose-dependent manner, whereas the inhibition by benzo(a)pyrene was not dose-dependent (Fig. 2). The antagonistic action of α-naphthoflavone strongly confirms the involvement of progesterone receptors in progesterone signaling resulting in 11α-progesterone hydroxylase induction.

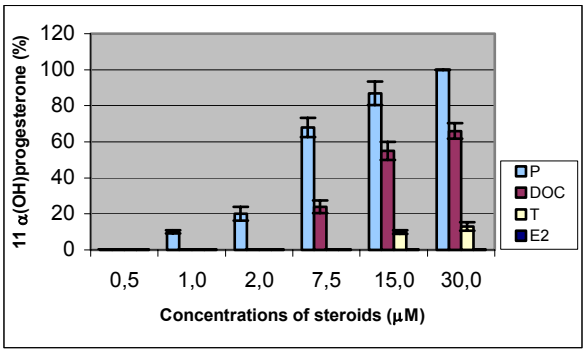


Fig. 1. Dose dependence of progesterone-hydroxylase induction by steroids.

In Fig 1 we can see that after growing for 18 hours *R. nigricans* was incubated for additional 2 hours with steroids of concentrations as indicated. Subsequently, a hydroxylation activity test was performed using progesterone as substrate. Results are presented in percent of conversion of progesterone into 11α-hydroxyprogesterone where the maximal conversion obtained by 30 μM progesterone was defined as 100%. P, progesterone; DOC, deoxycorticosterone; T, testosterone; E2 estradiol

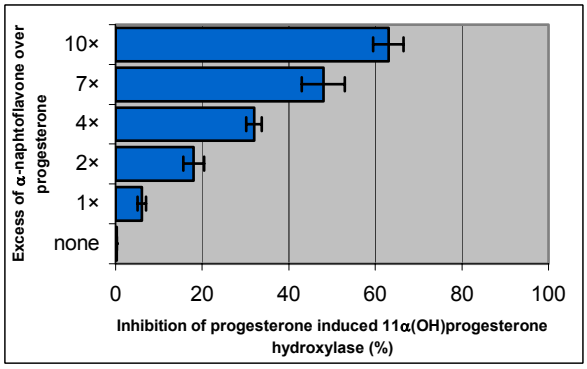


Fig. 2 A. Inhibition of progesterone induction of progesterone hydroxylase with simultaneous addition of indicated excesses of α-naphthoflavone

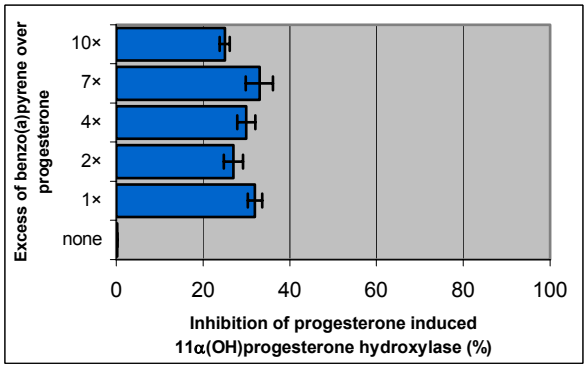
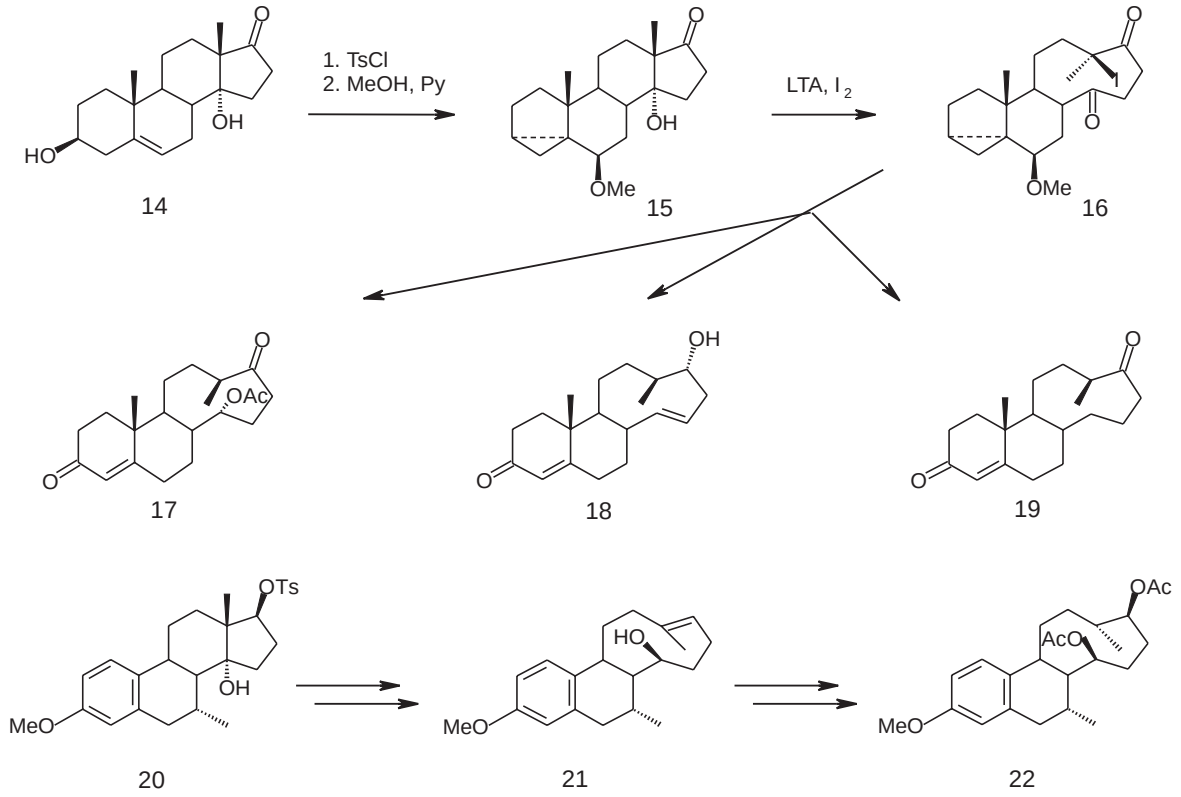


Fig. 2 B. Inhibition of progesterone induction of progesterone hydroxylase with simultaneous addition of indicated excesses of benzo(a)pyrene.

R. nigricans was grown for 18 hours and for additional 2 hours with 15 μM progesterone alone or with simultaneous addition of indicated excesses of (Fig. 2 A) α-naphthoflavone and (Fig. 2 B) benzo(a)pyrene. Hydroxylation activity test was performed using progesterone as substrate and the yield of 11α-hydroxyprogesterone determined. Results are presented in percent of inhibited progesterone-induced hydroxylase activity.

REFERENCES

1. Breskvar K., Ferencak Z., Hudnik-Plevnik T.: J. Steroid Biochem. Mol. Biol. *52*, 271 (1995).
2. Zakelj-Mavric M., Belic I.: J. Steroid Biochem. *28*, 197 (1987).
3. Hudnik-Plevnik T., Cresnar B.: J. Steroid Biochem. *35*, 749 (1990).
4. Lenasi H., Hudnik-Plevnik T.: Arch. Biochem. Biophys. *330*, 80 (1996).



-double bond, which both proceeded stereoselectively, to provide compound 22 as the only isomer.

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

SEMISYSTEMATIC NOMENCLATURE OF BRASSINOSTEROIDS

MARCO ANTÓNIO TEIXEIRA ZULLO[a], LADISLAV KOHOUT[b]

[a]Instituto Agronômico (IAC), P.O. Box 28, 13001-970 Campinas, SP, Brazil, [b]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
E-mail: mzullo@iac.sp.gov.br

The assignment of a trivial name to a natural product has the advantage of concentrating all the structural, including stereochemical, information in a single, or at most a few, simple word(s), since it is unique to this compound, but without knowing the compound one cannot advance these information. On the other hand, its systematic name carries all structural and stereochemical information, but is usually too long for being frequently used. The trivial name of a brassinosteroid is either derived from the plant source it was first isolated or detected (brassinolide, from *Brassica napus* L.; castasterone, from *Castanea crenata* Sieb. et Zuck; dolicholide and dolichosterone, from *Dolichos lablab* Adans.; typhasterol, from *Typha latifolia* G. F. W. Mey.; teasterone, from *Thea sinensis* L.; secasterone, from *Secale cereale* L.), either obtained by addition of adequate prefix(es) to the name of a previously known brassinosteroid (e.g. 28-homobrassinolide, 28-norcastasterone, 25-methyldolichosterone, 3-epi-2--deoxy-25-methyldolichosterone, etc.). More than 50 known brassinosteroids are 3-oxygenated (22*R*,23*R*)-5α-cholestane--22,23-diols, of plant origin, bearing alkyl or oxy substituents, conjugated or not to sugars or fatty acids[1]. This general structural feature allows the proposition of semisystematic names to the brassinosteroids, in which (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-5α-campestane, of trivial name 6-deoxocastasterone, is considered the functional parent compound and is named brassinostane or brassinane. The closely related compounds of trivial names, 6α-hydroxycastasterone, castasterone and brassinolide, would then be named 6α-brassinostanol, brassinostanone and brassinostanolactone, respectively, or 6α-brassinol, brassinone and brassinolide. The semisystematic names of the other members of the brassinosteroid family shall be given according to the established rules for naming natural products[2]. The use of these semisystematic names would avoid some mistakes in assigning trivial names to the brassinosteroids and the unpractical constant usage of their systematic names.

This work was supported by the grant No. A4055204 (GA CR) and research project Z4 055 905.

REFERENCES

1. Zullo M. A. T., Kohout L., De Azevedo M. B. M.: Plant Growth Regul. *39*, 1 (2003).
2. IUPAC: Pure Appl. Chem. *61*, 1783 (1999).