UNIVERSITY OF SURREY

An international multidisciplinary symposium

The Hyacinthaceae: Chemistry, Pharmacology, Taxonomy and Ethnobotany

Department of Chemistry, University of Surrey, UK

14 – 15 June 2012

Programme and Abstracts

IAS Institute of Advanced Studies

The Hyacinthaceae: Chemistry, Pharmacology, Taxonomy and Ethnobotany

An international multidisciplinary symposium

Welcome Message

I am delighted to welcome all participants to the symposium *The Hyacinthaceae: Chemistry, Pharmacology, Taxonomy and Ethnobotany.* Plant research covers a very wide field, and in this symposium researchers investigating all aspects of the important plant group, the Hyacinthaceae, from their taxonomy, their horticultural potential, their uses by traditional communities to their potential as drug candidates and their chemistry will be presenting their work.

We have put together a programme of speakers covering all aspects of research into the Hyacinthaceae that I hope you will enjoy. We also have an important poster session which includes posters showing research into the Hyacinthaceae and other plant families. We have researchers from fourteen countries presenting their work making this a truly international multidisciplinary event. We have an interesting social programme including a welcome get-together on the Wey River, dinner at a 16th century Surrey inn, the Withies Inn, and a visit to the Royal Horticultural Society Gardens at Wisley.

I would like to thank the Institute of Advanced Studies for their generous grant to enable this meeting to take place and support from the University. My committee and I hope you will enjoy your time at the University of Surrey and in Guildford.

I hope that discussions arising at the Symposium will generate long lasting fruitful collaborations.

Dulcie Mulholland
Symposium Chair

International Advisory Committee:

Professor Neil Crouch (SANBI, South Africa), Dr Fabio Fusi (University of Siena, Italy)
Professor Wolfgang Wetschnig, (University of Graz, Austria).

Local Organising Committee: Natural Products Research Group

Moses Langat (Transport)
Jaspreet Sihra (Oral presentations)
Watcharee Waratchareeyakul (Session 1)
Linda Langat (Session 3)
Catherine Waller (Poster session)
Dorota Nawrot (Catering)
Alaa Alqhatani (Session 2)
Alfred Thumser

Plant photographs courtesy of Neil Crouch.

With thanks to Mirela Dumic and Rita Dunford for administrative assistance.

The Hyacinthaceae: Chemistry, Pharmacology, Taxonomy and Ethnobotany

Programme

Programme

14 June 2012

08:30	**09:00**	**Registration and Coffee**
09:00	09:10	Welcome by Professor Steve Williamson, Deputy Vice Chancellor (Research)
09:10	09:40	The Hyacinthaceae: A Horticultural Perspective, Paul Cumbleton, RHS Gardens, Wisley
09:40	10:20	The Hyacinthaceae: a taxonomic overview, Wolfgang Wetschnig, University of Graz
10:20	11:00	Iminosugars as a systematic character in Hyacinthaceae, Geoffrey Kite, Royal Botanic Gardens, Kew
11:00	**11:15**	**Coffee break**
11:15	11:55	South African Hyacinthaceae in Ethnomedicine, Neil Crouch, SANBI
11:55	12:35	The Phytochemistry of the Southern African Hyacinthaceae, Dulcie Mulholland, University of Surrey
12:35	13:00	Novel triterpenoid derivatives from *Eucomis bicolor* (Hyacinthaceae: Hyacinthoideae), Jaspreet Sihra, University of Surrey
13:00	**14:00**	**Lunch**
14:00	14:40	Biogeographic and phylogenetic patterns of subfamily Urgineoideae (Hyacinthaceae) as inferred by analysis of plastid DNA data, Martin Pfosser, Biocenter Linz
14:40	15:05	COX-2 specific inhibitors from *Ledebouria ovatifolia*, *Ledebouria socialis* and *Drimiopsis burkei,* Catherine Waller, University of Surrey
15:05	15:45	An overview of the cyclo-oxygenases and inhibition by plant-based products, Alfred Thumser, University of Surrey
15.45	16.20	Taxonomy and Systematics of Fam. Hyacinthaceae subfam. Ornithogaloideae Mario Martinez Azorin, Universidad de Alicante Apdo. Alicante, Spain
16.20	18:00	Poster session and coffee
18:30	**21:30**	**Dinner at the Withies Inn, Compton Village**

15 June 2012

09:00	09:40	Pharmacology of the Hyacinthaceae family, Fabio Fusi, University of Siena
09:40	10:20	Urgineoideae of Madagascar, Walter Knirsch, University of Graz
10:20	11:00	Screening in *Silico* – Emergence of the Virtual Human, Nick Plant, University of Surrey
11:00	11:15	General Discussion. The Way Forward
11:15	11:30	Depart for RHS Gardens at Wisley (transport organised from the University)
11:45	**13:00**	**Lunch at RHS Gardens at Wisley**
13:00	15:00	Garden Tour
15:30	18:00	Drinks and meal in a riverside pub (Optional and weather dependant)

Our Speakers

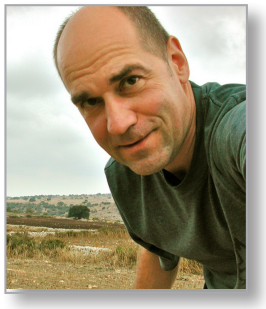
Paul Cumbleton qualified in Botany with Zoology from the University of Reading and has worked for 22 years as a scientific officer for the Agricultural Development and Advisory Service (ADAS), mainly involved in a variety of horticultural and agricultural trials. For the past ten years he has worked for the Royal Horticultural Society as team leader for the Alpine Department at RHS Gardens at Wisley. Paul has a keen interest in alpine plants in general a special passion for the genus *Pleione* (see website: www.pleione.info) and for the winter-growing bulbous flora of South Africa.

Wolfgang Wetschnig graduated at the Karl-Franzens-University, Graz (Austria) in 1982. He is working as a.o.Prof. at the Institute of Plant Sciences of this University since 1995 were he is researching and teaching plant systematics. In 1987 he started investigating Hyacinthaceae during a 6-month fellowship at the University of Cape Town and at the Kirstenbosch Botanical Garden. His main interests are the taxonomy and morphology (with special regards to seed morphology) of Hyacinthaceae.

Geoffrey Kite joined the Biochemistry section of the Royal Botanic Gardens Kew in 1986, having completed a PhD in the evolution of intracellular symbiosis in algae following a first degree in Botany, both at the University of London. Since then, he has himself evolved into a mass spectroscopist as, by necessity, he became responsible for mass spectrometry equipment as it was acquired. He has applied GC-MS and LC-MS to various research projects in systematic phytochemistry and has authored 137 papers. Now in the Sustainable Uses Group at Kew he increasingly assists with analytical aspects of projects concerned with the authentication of herbs and extracts, and the search for new uses of plants.

Neil R. Crouch has a PhD in Botany. He heads up the Ethnobotany Unit of the South African National Biodiversity Institute (SANBI) in Durban, South Africa where he is also an Honorary Professor in the School of Chemistry at the University of KwaZulu-Natal. His research interests focus primarily on medicinal plants and their potential in drug development. He has collaborated extensively and published over 150 peer-reviewed scientific papers, and authored or coauthored a range of books, including Guide to Succulents of Southern Africa (2009), and Ferns of Southern Africa. A Comprehensive Guide (2011). His interests in the Hyacinthaceae include their chemistry, traditional uses, systematics, taxonomy and horticulture, leading him to discover and describe several new species during the past decade.

Our Speakers



Martin Pfosser graduated at the University of Agriculture, Vienna (Austria). Fellowship of the Japan Society for the Promotion of Science at Tohoko University. Assistant Professor at the Institute of Microbiology and Genetics and the Institute of Botany and Evolutionary Biology of Vienna University. Since 2003 Head of Botany Department, Biocenter Linz of the Museum of Upper Austria. Main interests are in molecular phylogeny and biogeography of Hyacinthaceae.

Alfred Thumser completed his PhD degree in Drug Metabolism at the University of Cape Town, South Africa, and subsequently embarked on two extensive post-doctoral research posts at the University of Southampton, United Kingdom, and Rutgers University, New Jersey, USA, where he worked on fatty acid metabolism. After his post-doc in New Jersey he returned to the United Kingdom for an academic post at the University of Surrey. His current research interests focus on metabolic processes (metabolomics) and enzyme-based fuel cells.

Mario Martinez Azorin obtained a PhD in Biology is June 2008 on the topic "Taxonomy and systematics in Ornithogaloideae". He has held Postgraduate and Postdoctoral Fellowships at Kew gardens, Kirstenbosch (Cape Town) and Rhodes University in Grahamstown.

Fabio Fusi graduated in 1991 in Pharmaceutical Chemistry and Technology (Pharmacy) and obtained a Ph.D. in Pharmaceutical Sciences (1994) from the University of Siena, Italy, where he presently holds a researcher position in the Department of Neurosciences. His experimental research is focussed on the in vitro activity of natural compounds on vascular smooth muscle ion channels. He has worked in Dublin, Sofia, Springfield (IL, USA), and Cambridge. He is a Member of the Italian Pharmacological Society and he teaches pharmacological assays and dosages at the Faculty of Pharmacy (course in Pharmaceutical Chemistry and Technology). He has published 53 papers and 28 abstracts in International Journals.

Our Speakers

Walter Knirsch obtained a M.D. degree from the University of Graz in 1989 and has been an ophthalmologist since 1989. In 2007 he obtained a Bachelor's degree and in 2012 a Master's degree in Botany from the University of Graz. He has made several trips to Madagascar, working on the Bulbophyllum project with the University of Vienna.

Nick Plant undertook both his undergraduate and postgraduate studies at the University of Nottingham, where he read Biochemistry and Genetics. He was then appointed as a GlaxoSmithKline Fellow at the University of Surrey, where he continues to work presently as a Reader in Molecular Toxicology. Dr. Nick Plant has 15 years experience researching the coordination of cellular responses to xenobiotic challenge. His research has focussed on members of the super-family of nuclear receptors, which act as ligand-activated transcription factors, sensing their cellular surroundings and coordinating network responses to any disruption of homeostasis. He currently sits on the Committee for Toxicology of Consumer Products, Foodstuffs and the Environment, and the MHRA Pharmacovigilance Expert Advisory Group.

Catherine Waller (left) and **Jaspreet Sihra** (right) are students in the Natural Products Research Group at the University of Surrey. They are both in their final stages of PhD thesis preparation.

The Hyacinthaceae: Chemistry, Pharmacology, Taxonomy and Ethnobotany

Lecture Abstracts

SESSION 1: Session Chair: Dr Alfred Thumser

The Hyacinthaceae: A Horticultural Perspective

Paul Cumbleton, RHS Gardens at Wisley, Surrey, UK

This presentation aims to provide a context for the papers which are to follow. It will be in the form of a photographic-based presentation showing some of the key species currently familiar within horticulture and some of those not yet common in cultivation but which have clear potential. The economic importance of some species and on some of the more scientific aspects of the work the Royal Horticultural Society will be discussed. The plants we work with are of more than just scientific or financial interest but have their own intrinsic beauty that has endeared them to gardeners around the world. Hopefully the presentation will reveal the attraction of at least some of them.

The Hyacinthaceae: A Taxonomic Overview

Wolfgang Wetschnig, Institute of Plant Sciences, University of Graz, Austria

The taxonomic history of the Hyacinthaceae started with three genera and ended up with more than seventy. Especially in the last decade the generic concepts of the subfamilies Ornithogaloideae and Urgineoideae differed substantially. In this talk I want to summarize and discuss the recent taxonomic concepts with special emphasis on the subfamily Urgineoideae.

Iminosugars as a systematic character in Hyacinthaceae

Geoffrey Kite, Royal Botanic Gardens Kew, Surrey, UK

Iminosugars have been isolated from species in several plant families, including Hyacinthaceae, and they continue to excite interest as potential drug candidates. Hyacinthaceae have proved to be a rich source of iminosugars with new examples having been isolated from species in several genera, including *Hyacinthoides, Hyacinthus, Muscari* and *Scilla*. Systematic studies on the occurrence of iminosugars in some other families, such as Araceae and Euphorbiaceae, have revealed that species accumulating these compounds form clusters on molecular phylogenies generated from DNA sequence data, suggesting that iminosugar accumulation may be a useful systematic character. The understanding of relationships of, and within, Hyacinthaceae have been advanced significantly by molecular studies, so it seems an opportune moment to review the occurrence of iminosugars in the family and related taxa within a phylogenetic framework. Data on species accumulating iminosugars will be compiled from published literature, re-examination of the in-silico analytical collections at RBG Kew and new analyses of key taxa. The distribution of taxa accumulating iminosugars will be mapped on published molecular phylogenies to assess the systematic potential of this chemical character in Hyacinthaceae and closely related families.

Southern African Hyacinthaceae in Ethnomedicine

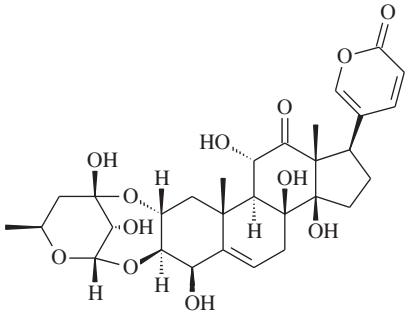
Neil Crouch, Ethnobotany Unit, South African National Biodiversity Institute, South Africa / School of Chemistry, University of KwaZulu-Natal, Durban, South Africa

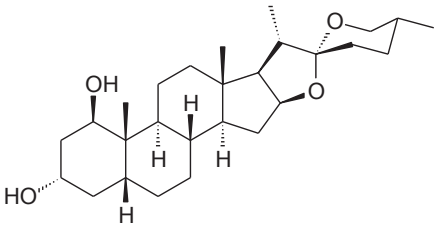
Within the Flora of Southern Africa (FSA) region three subfamilies of the Hyacinthaceae are distributed widely in both winter- and summer-rainfall regions. However, it is within the latter distribution range that bulbs of this large family are extensively used in ethnomedicine, ranking amongst the most popular species in trade. The historical usage of plants is discussed, as is their role in both healing and homicide. Findings are presented in relation to infrafamilial arrangements, exploring chemotaxonomic relationships, and thus the potential for rational and efficient drug discovery. Concerns relating to the unsustainable utilisation of members of the Hyacinthaceae are discussed, as are approaches to their in *situ* and *ex situ* conservation.

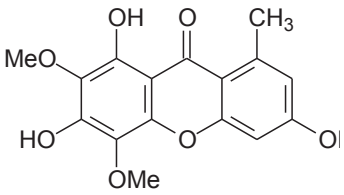
The Phytochemistry of the Southern African Hyacinthaceae

Dulcie Mulholland, Natural Products Research Group, Department of Chemistry, University of Surrey, UK

Investigations into the phytochemistry of ethnomedicinally important Hyacinthaceae from Southern Africa over the past twelve years will be described. The Hyacinthoideae have yielded a range of homoisoflavonoids, xanthones, norlignans and complex triterpenoid derivatives and we have shown that homoisoflavanones also occur in the Urgineoideae and Ornithogaloideae. The Urgineiodeae have yielded a range of bufadienolides and their complex glycosides, and the Ornithogaloideae, largely phytosteroidal compounds. Synthesis of norlignans with anti-inflammatory potential will be discussed. Some examples of novel compounds isolated that will be discussed are shown below.



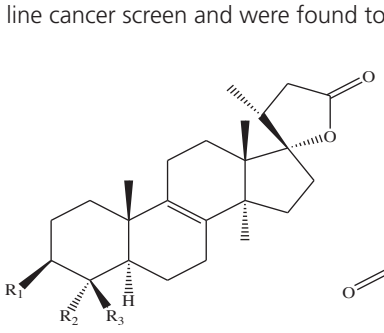




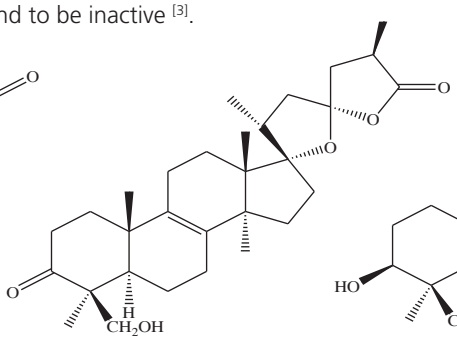
Novel triterpenoid derivatives from *Eucomis bicolor* (Hyacinthaceae: Hyacinthoideae)

Jaspreet K. Sihra [a], Dulcie A. Mulholland [a], Moses K. Langat [a], Neil R. Crouch [b] and Jean-Marc Nuzillard.[c].
[a] Natural Products Research Group, Division of Chemical Sciences, University of Surrey, UK
[b] Ethnobotany Unit, South African National Biodiversity Institute, Durban, South Africa/School of Chemistry, University of KwaZulu-Natal, Durban, South Africa, [c] Institute of Molecular Chemistry, University of Reims, France

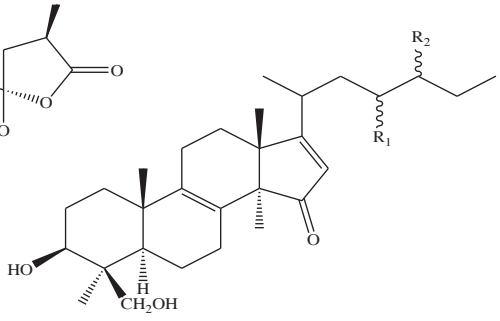
The genus *Eucomis* has long been one of the most highly regarded sources of ethnomedicines in southern Africa and is used traditionally to treat inflammation, urinary diseases, colic, hangovers, syphilis, fractures, backache, rheumatism and teething [1]. *Eucomis bicolor* Bak. is used as a purgative and for the treatment of colic [1]. Eucomis bicolor has been previously investigated phytochemically, however, as the Hyacinthoideae are known to have significant geographical chemical variation [2], *Eucomis bicolor* collected from a different location was reinvestigated. The analysis of the dichloromethane extract of the whole plant of *Eucomis bicolor* yielded twelve compounds, including five novel compounds (1-5), which were submitted to the NCI to be tested on a 60-cell line cancer screen and were found to be inactive [3].



Compound 1: R_1 = =O, R_2 = H, R_3 = CH_3
Compound 2: R_1 = OH, R_2 = R_3 = CH_2OH



Compound 3



Compound 4: R_1 = R_2 = OH
Compound 5: R_1 = OH, R_2 = =O

[1] Koorbanally, C., Crouch, N. R. and Mulholland, D. A., 2006. The phytochemistry and ethnobotany of the southern African genus *Eucomis* (Hyacinthaceae: Hyacinthoideae). Phytochemistry: Advances in Research, 69-85.
[2] Mulholland, D. A., Crouch, N. R., Koorbanally, C., Moodley, N., Pohl, T., 2006. Infraspecific chemical variation in *Scilla zebrina* (Hyacinthaceae). Biochem. Syst. Ecol. 34, 251-255.
[3] http://dtp.nci.nih.gov/branches/btb/ivclsp.html

The Hyacinthaceae: Chemistry, Pharmacology, Taxonomy and Ethnobotany

An international multidisciplinary Symposium

SESSION 2: Session Chair: Prof Neil Crouch

Biogeographic and phylogenetic patterns of subfamily Urgineoideae (Hyacinthaceae) as inferred by analysis of plastid DNA data

Martin Pfosser, Biocenter Linz, Austria

Subamily Urgineoideae of family Hyacinthaceae shows a wide distribution area spanning large parts of the African continent, Madagascar, the Arabian Peninsula, India, and the Mediterranean region. Phylogenetic analysis of various plastid DNA regions including the highly variable non-coding trnL-F and ycf6 regions all point to a South African origin for the whole subfamily. The mode of evolution in Urgineoideae leading to its present day distribution is far less clear. For example, until recently all members of the subfamily present in Madagascar have been included within the genus *Rhadamanthus* with most representatives found on mainland Africa. This decision was based mainly on cytological and morphological characters shared between both groups of species. Molecular data now suggest a single colonization event for the whole Malagasy alliance, and furthermore, this group appears to be not directly related to members of the genus *Rhadamanthus* of mainland Africa. On the other hand, colonization of the Mediterranean region shows a different pattern, involving at least two independent migration scenarios. In this presentation we intend to summarize our current view of the mode of evolution in this large subfamily.

COX-2 specific inhibitors from *Ledebouria ovatifolia, Ledebouria socialis* and *Drimiopsis burkei* (Hyacinthaceae: Hyacinthoideae)

Catherine Waller,[a] Neil. R. Crouch,[b,c] Alfred E. Thumser,[a] Moses K. Langat,[a] Dulcie A. Mulholland[a]
[a] Natural Products Research Group, University of Surrey, UK. b School of Chemistry, University of KwaZulu-Natal, Durban, South Africa. [c] Ethnobotany Unit, South African National Biodiversity Institute, Durban, South Africa

The phytochemical investigation of *Ledebouria ovatifolia* and *Ledebouria socialis* yielded nine novel compounds. One homoisoflavanone **1** was isolated from *Ledebouria socialis*. From *Ledebouria ovatifolia*, a dihydro-chalcone **2**, a cycloartane derivative **3**, five homoisoflavanones **4, 5, 6, 8, 9**, and one xanthone **7**, were isolated. These included two isomers of 2-hydroxy-7-O-methyl-scillascillin, **4** and **5**, which were separated by acetylation and were identified using NMR spectroscopy and excitation circular dichroism experiments.

The above compounds were isolated along with twenty-three known compounds including one polyhydroxylated furan derivative, polybotrin, which had not previously been isolated from the Hyacinthaceae family. Concurrently, the norlignan (*E*)-hinokiresinol (isolated previously from *Drimiopsis burkei* (Du Toit et al., 2005) and derivatives were synthesised due to anti- inflammatory activity seen against the cyclooxygenase enzyme. Homoisoflavanones are also known to show anti-inflammatory activity (Della Loggia et al., 1989). The homoisoflavonone derivatives and synthesised compounds from the three plants were screened against COX-1 and COX-2 enzymes, compounds (*E*)-3-(3,4- dihydroxybenzylidene)-7-hydroxy-5-methoxychroman-4-one, 5,7-dihydroxy-3-(4'- hydroxybenzyl)-4-chromanone and 5,7-dihydroxy-3-(4'-methoxybenzyl)-chroman-4- one inhibited and showed selectivity for COX-2 at 10μM. (E)-Hinokiresinol had an IC50 of 1μM and at least twenty fold selectivity for COX-2 over COX-1.

Della Loggia, R., Del Negro, P., Tubaro, A., Barone, G., Parrilli, M. (1989), *Planta Medica*, 55, 587- 588. Du Toit, K., Elgorashi, E., Malan, S., Drewes, S., van Staden, J., Crouch, N., Mulholland, D.A., (2005), *Bioorganic and Medicinal Chemistry*, 13, (7), 2561-2568.

An overview of the cyclo-oxygenases and inhibition by plant-based products

Alfred Thumser, Dept. of Biochemistry and Physiology, University of Surrey, UK

The talk will provide an overview of the biochemistry and physiology of the cyclo-oxygenase (COX) enzymes and their role in inflammatory processes, in particular the activation of arachidonic acid. Subsequently I will cover a COX assay that we use at Surrey, followed by inhibition data obtained with isolated plant products with the COX-1 and COX-2. Finally, time permitting, a discussion on moving this work forward in developing realistic drug targets.

Taxonomy and Systematics of Fam. Hyacinthaceae subfam. Ornithogaloideae

Mario Martinez Azorin, Depto. Ciencias Ambientales y Recursos Naturales CIBIO (Instituto de la Biodiversidad) Universidad de Alicante Apdo. Alicante, Spain

The taxonomic arrangement within subfamily Ornithogaloideae (Hyacinthaceae) has been a matter of controversy in recent decades. Several new taxonomic treatments have been proposed, based exclusively on plastid DNA sequences, and these have resulted in classifications which are to a great extent contradictory. Some authors have recognized only a single genus *Ornithogalum* for the whole subfamily, including 250-300 spp. of very variable morphology, whereas others have recognized many genera. In the latter case, the genera are inevitably much smaller and they are better defined morphologically. However, some are not monophyletic as circumscribed in the past.

Our phylogenetic analyses of Ornithogaloideae were based on nucleotide sequences of four plastid regions (*trnL* intron, *trnL-F* spacer, *rbcL* and *matK*) and a nuclear region (ITS). Eighty species covering all relevant taxonomic groups previously recognized in the subfamily were sampled. The molecular data were compared with a matrix of 34 morphological characters.

Combinations of plastid and nuclear data yielded phylogenetic trees which are better resolved than those obtained with any plastid region alone or plastid regions in combination. Three main clades are found, corresponding to the previously recognised tribes *Albuceae, Dipcadieae* and *Ornithogaleae*. In these, up to 19 clades are described which are definable by morphology and biogeography. These mostly correspond to previously described taxa, though some need recircumscription. Morphological characters are assessed for their diagnostic value for taxonomy in the subfamily.

On the basis of the phylogenetic analyses, 19 monophyletic genera are accepted within Ornithogaloideae: *Albuca, Avonsera, Battandiera, Cathissa, Coilonox, Dipcadi, Eliokarmos, Elsiea, Ethesia, Galtonia, Honorius, Loncomelos, Melomphis, Neopatersonia, Nicipe, Ornithogalum, Pseudogaltonia, Stellarioides* and *Trimelopter.* Each of these has a particular syndrome of morphological characters making these genera more homogeneous in morphology and hence easier to recognize and circumscribe.

The Hyacinthaceae: Chemistry, Pharmacology, Taxonomy and Ethnobotany

Lecture Abstracts

SESSION 3: Session Chair: Professor Wolfgang Wetschnig

Pharmacology of the Hyacinthaceae family

Fabio Fusi, University of Siena, Italy

Natural products continue to provide a diverse and unique source of bioactive lead compounds for drug discovery. Although estimates vary depending on the definition of what is considered a natural product-derived drug, it is safe to say that between 25% and 50% of currently marketed drugs owe their origins to natural products. In addition to their use as drugs or lead compounds for drug development, natural products have also played a key role in drug discovery by serving as chemical probes. In fact, the diversity and complexity of natural products makes them able to target biological macromolecules, often in a highly selective fashion. In this scenario, the Hyacinthaceae family represents a still under-investigated group of plants. Constituents of the Hyacinthaceae have provided compounds with great potential as anti-inflammatories. The recently described vascular myorelaxing activity of isolates from South African Hyacinthaceae, however, has opened a new chapter in the pharmacology of these natural products. Scillascillin-type homoisoflavonoids, by exhibiting both antispasmodic and spasmolytic activity via a negative modulation of plasmalemmal Ca2+ influx responsible for the contraction of the vascular musculature, lead to the conclusion that these compounds may represent novel vasodilators, or at least provide scaffolds for the design of novel vasoactive agents. Accordingly, the vasodilating effect of homoisoflavanoids validated their inclusion in traditional preparations to treat ailments associated with vascular dysfunction. An expanding exploration of isolates from the Hyacinthaceae family is therefore advocated.

Urgineoideae of Madagascar

Walter Knirsch, University of Graz, Institute of Plant Sciences, Austria

Assessment of the taxonomic position of Malagasy Urgineoideae in relation to the South African genus *Rhadamanthus* Salisb. and to the other genera within the subfamily Urgineoideae. Investigation and evaluation of their taxonomic status using one plastid DNA region: trnCGCA-ycf6. Presentation of the species belonging to the genus Rhodocodon.

Screening in Silico – Emergence of the virtual human

Nick Plant, Centre for Toxicology, University of Surrey, UK

At present, the pharmaceutical industry is stuck in an escalating spiral of costs for the discovery and development of drugs. These increased costs would be burdensome enough without the added complication that many development drugs never make it to market, often failing at the stage of clinical trials when many millions of dollars has already been spent. In an attempt to reduce costs, and to make smart decisions earlier, much work has been focussed in recent years on the discovery/development border line; this work is aimed at improving the identification of those novel chemical entities most likely to make it to market.

At the University of Surrey we have focussed on the development of computer modelling approaches to aid in the correct selection of novel chemical entities for development. These models are targeted to increase the robustness of extrapolation from in *vitro* assays to pre-clinical species, and from pre-clinical species to human responses. This presentation will cover the basic aspects of such computational approaches, building towards the ultimate aim of the in *silico* human.

The Hyacinthaceae: Chemistry, Pharmacology, Taxonomy and Ethnobotany

Poster Abstracts

1. New Pentacyclic Oxindole Alkaloids from Malaysian *Uncaria longiflora* var. pteropoda Miq. (Ridsd.)

Rohaya Ahmad[1,2] and Fatimah Salim[2]
[1]Atta-ur-Rahman Institute of Natural Product Discovery (RiND)
Universiti Teknologi MARA, Malaysia
[2]Faculty of Applied Sciences, Universiti Teknologi MARA, Malaysia

Uncaria longiflora var. pteropoda (Miq.) is a subspecies belonging to the genus *Uncaria* of the family Rubiaceae. Previous work on the leaves extract of the Malaysian plant by Yeoh *et al*., in 1966 and Kam et al. in 1992 had afforded two pentacyclic oxindole alkaloids, isopteropodine and pteropodine. Our present phytochemical study on the stems extracts of the plant have yielded four new pentacyclic oxindole alkaloids, rauniticine-allo-oxindole B (1), rauniticinic-allo acid B (2), 17β-hydroxyisoformosanine (3) and 17β-hydroxyformosanine (4) along with five known alkaloids, pteropodine, isopteropodine, uncarine F, speciophylline and isopteropodic acid. The structures of the new compounds and their relative configurations were determined using various spectroscopic methods including 1D and 2D NMR and comparison with literature.

2. Phylogeographic relationships within *Dipcadi* Medik. and their possible migration and colonization routes

Michael Pinter, University of Graz, Austria

Whereas most of the genera within the Hyacinthaceae have a geographic restricted distribution area, the genus *Dipcadi* Medik. is the one with the widest, that includes Europe, Africa and Asia. It is native to the western Mediterranean parts of Europe and Northern Africa, the sub-Saharan region, the Arabian Peninsula, Socotra, Madagascar and India. There were about 140 Taxa described and according to the wide distribution area and the high number of taxa, there dominates taxonomic chaos. Centres of highest diversification within the genus are out of doubt India, South Africa and Madagascar. DNA sequence analysis showed that the Malagasy species have not reached the island by a single colonization event from the mainland of Africa; there must have been at least two events which were followed by fast radiation. There is a strict context with the theory of plate tectonic by A. WEGENER and the relationships within *Dipcadi* on the one hand for species located southwards the Arabian Peninsula and with a possible migration route, that linked India and the Mediterranean Regions on the other. The Arabian Peninsula in the analysis is divided into an independent and an East Africa / Madagascar / Arabian Peninsula clade, that is not closely related to the European and Indian species. There was no correlation found between the Indian and the Malagasy species, as it was for members of the other subfamilies. So far, data have been analysed for a high quantity of different taxa, that now refute the theory of a single colonization event of Madagascar.

The Hyacinthaceae: Chemistry, Pharmacology, Taxonomy and Ethnobotany

Poster Abstracts

3. Phytochemistry of *Urginavia altissima* (Hyacinthaceae)

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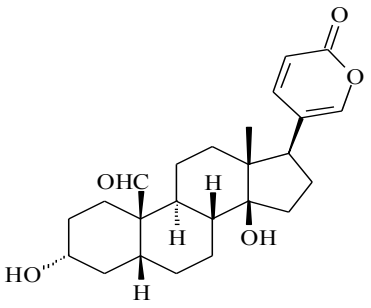
The bulbs of *Urginavia altissima* have yielded several compounds including three new bufadienolides, 3-*epi*-19-oxobufalin (**1**), the 3-epimer of 19-oxobufalin previously isolated from the Chinese traditional drug Ch'an Su, a product of skin secretions from the Chinese toad Bufo gargarizans Cantor[1], 3α,11α,14β-trihydroxybufa-4,20,22-trienolide (**2**), the 3-keto derivative of which has been isolated previously from the bulbs of closely related *Urginea maritima* and *Urginea lydenburgensis*[2], and a possible artefact, 3α-ethoxy-11α,14β-dihydroxybufa-4,20,22-trienolide (**3**). A new di-unsaturated hydroxylated fatty acid ethyl ester (**4**) together with known compounds were also isolated from the plant.



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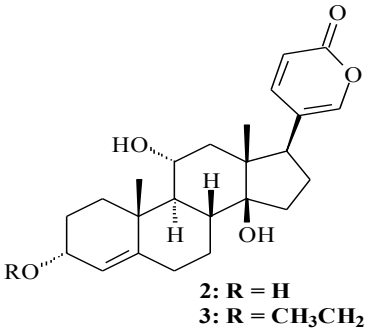
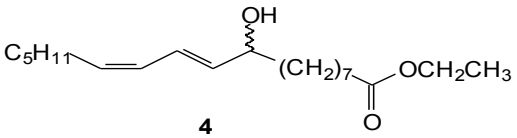


2: R = H
3: R = CH_3CH_2



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[1] Nogawa, T. *et al.*, 2001. *J. Nat. Prod.* 64, 1148-1152
[2] Gao, H. *et al.*, 2011. *Nat. Prod. Rep*. 28, 953-969

4. A bufadienolide glycoside from *Rhodocodon* aff. calcicola (Hyacinthaceae)

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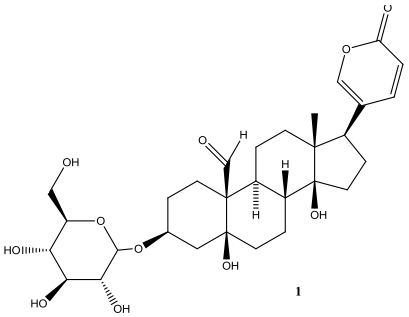
[1]Natural Products Research Group, Chemical Sciences, Faculty of Health and Medical Sciences, University of Surrey, [2] Institute of Plant Sciences, University of Graz, Austria

The phytochemistry of the *Rhodocodon* genus (Hyacinthaceae) has not been investigated previously. In this study we report on the isolation of several compounds from the ethanol extract of the bulbs of *Rhodocodon* aff. *calcicola* (collections 04474 & 04476). One of the compounds isolated was the bufadienolide glycoside, hellebrigenin β-glucoside (**1**), reported previously to occur in *Urginea altissima* collected from Kenya. [1] However, its NMR data have not been reported before. The structures of the compounds were determined using NMR studies. Bufadienolides are widely used in traditional remedies for the treatment of several ailments, such as infections, rheumatism, inflammation and disorders associated with the central nervous system.[2]



1

[1] Shimada, K. Umezawa, K. Nambara,T. and Kupchan, S. M. 1979. *Chem. Pharm. Bull*. 27, 3111 - 3114
[2] Goel, A. and Ram, V. J. 2009. *Tetrahedron* 65, 7865-7913

5. Tetranortriterpenoid derivatives from *Xylocarpus rumphii*

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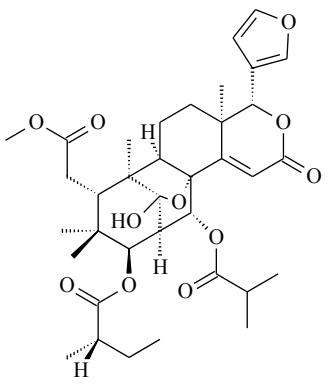
[4] Prince of Songkla University, Songkhla, Thailand, 90110

Limonoids, which are tetranortriterpenoid derivatives, commonly occur in the Meliaceae family, including the genus *Xylocarpus*.[1,2] Two compounds, the known xylorumphiin C (**1**) and the novel C-23 epimeric xylorumpholide B (**2**) were isolated from *Xylocarpus rumphii.* Column chromatographic separation of an acetylated fraction of impure **2** led to isolation of four acetylated derivatives of 2 (**2a, 2b, 2c** and **2d**). The structures of the compounds were determined using NMR, IR and MS analysis.



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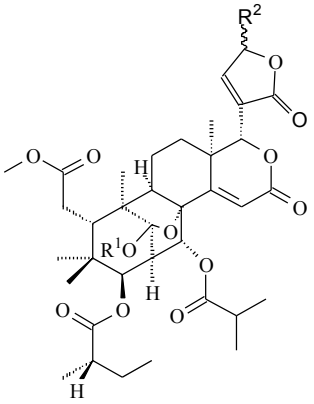

2 : $R^1 = H, R^2 = OH$
2a : $R^1 = H, R^2 = \alpha\text{-OAc}$
2b : $R^1 = H, R^2 = \beta\text{-OAc}$
2c : $R^1 = Ac, R^2 = \alpha\text{-OAc}$
2d : $R^1 = Ac. R^2 = \beta\text{-OAc}$

Compounds **1** and **2** have been submitted to the NCI 60 cancer cell line screen.

[1]Sarigaputi, C., Nuanyai, T., Teerawatananond, T., Pengpreecha, S., Muangsin, N. and Pudhom, K. (2010) J. *Nat. Prod*. 73, 1456-1459.
[2]Li, M.-Y., Yang, X.-B., Pan, J.-Y., Feng, G., Xiao, Q., Sinkkonen, J., Satyanandamurty, T. and Wu, J. (2009) J. *Nat. Prod*. 72, 2110-2114.

6. Determination of Absolute Configuration of (*5R,8R,9S,10R*)-15-acetoxy-2-oxo-*trans*-*ent*-cleroda-3,13-diene isolated from African *Croton sylvaticus* by Excitation Circular Dichroism

Moses K. Langat,[1] Neil A. Crouch[2,3] and Dulcie A. Mulholland[1]
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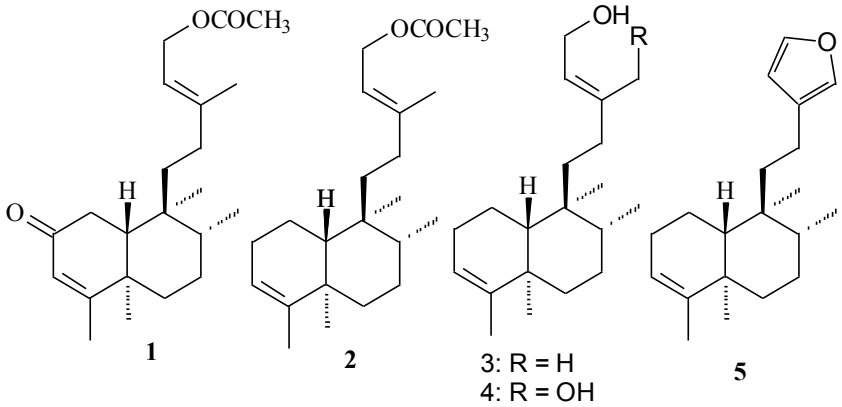
The biosynthesis of the clerodane class of diterpenoids produces two major enantiomeric subclasses of clerodanes (normal and *ent*-) with the normal clerodane subclass possessing H-10 in the α–orientation (10S) whereas the *ent*-clerodane subclass has H-10 in the β–orientation (10R). The two enantiomeric subclasses are usually distinguished based on the signs of their optical rotational values, with normal clerodanes giving +ve value whereas the *ent*-clerodane gives a –ve value.[1] The stem and leaves of *Croton sylvaticus* yielded several known compounds and five clerodane diterpenoids whose enantiomeric series could not be assigned using the sign of their optical rotational values. The absolute configuration of **1** was unambiguously assigned as (5R,8R,9S,10R)-15-acetoxy-2-oxo-trans-*ent*-cleroda-3,13-diene by comparison of its experimental excitation circular dichroism (ECD) spectrum with theoretical spectra obtained by density functional theory calculations of its normal and *ent*-clerodane isomers. The experimental ECD spectra of compounds **2, 3** and **4** were compared to that of **1**.



[1]Costa, M. et al., 1999. *Phytochemistry*. 50, 117-122

7. Coumarins from the Malagasy *Cedrelopsis rakotozafyi* (Ptaeroxylaceae)

Dorota Nawrot and Dulcie A. Mulholland, Department of Chemistry, University of Surrey, UK

*Cedrelopsis rakotozafy*i, local name Hazondranta, belongs to the Ptaeroxylaceae and was collected from Ampitsinjovagna, Irodo, Madagascar. The genus *Cedrelopsis* is endemic to Madagascar and comprises eight species, four of which have been examined phytochemically: *C. gracilis , C. microfoliata, C. grevei* and *C. longibracteata*. The plants which belong to the Cedrelopsis genus are widely used for folk remedies as febrifuges, fortifying tonics, relaxing and postnatal medications [**1, 2**]. They are also used to cure rheumatism and to treat sexually transmitted infections [**3**]. The plant *Cedrelopsis rakotozafyi* has been investigated and yielded seven compounds: a new coumarin, 8-hydroxy-7-methoxy-6-(2-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one along with four known coumarin derivatives and two known triterpenoids. No limonoids were isolated. Coumarins possess many biological activities: anti-bacterial, anti-coagulant, anti-mutagenic, anti-inflammatory, as well as capacity to inhibit platelet aggregation, reactive oxygen species (ROS) scavenging capacity and anti-HIV activity [**4**]. A recently isolated novel coumarin, from *Toddalia asiatica* shows a dual effect as a cell differentiating agent and apoptosis inducer in U-937 cells, suggesting the promising development of novel anti-leukemic agents [**4**].

[1] Gauvin, A., Ravaomanarivo, H., Smadja, J., 2004. Comparative analysis by gas chromatography-mass spectrometry of the essential oils from bark and leaves of *Cedrelopsis grevei* Baill, an aromatic and medicinal plant from Madagascar. J Chromatogr. A 1029, 279-282.

[2] Um, B.H., Lobstein, A., Weniger, B., Spiegel, C., Yice, F., Rakotoarison, O., Andriantsitohaina, R., Anton, R., 2003. New coumarins from *Cedrelopsis grevei*. Fitoterapia 74, 638-642.

[3] Mulholland, D.A., McFarland, K., Randrianarivelojosia, M., Rabarison, H., 2004. Cedkathryns A and B, pentanortriterpenoids from *Cedrelopsis gracilis* (Ptaeroxylaceae). Phytochemistry 65, 2929-2934.

[4] Coombes, P. H., Crouch, N., Ismail, F., Mulholland, D., A. Apoptosis and cell differentiation effects of Toddaculin, a natural coumarin from *Toddalia asiatica*, on U-937 cells. Phytomedicine, *in press*.

8. Chemical constituents of East European Forest species – The ForestSpecs project.

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Natural Products Research Group, Department of Chemistry, University of Surrey, UK

The development of modern and environmentally friendly products, materials and processes to make use of abundant sources of wood waste as starting material for value-added products is an important area of research. This work is part of the EU FP7 (FP7-KBB-2008-2B-227239) ForestSpecs project whose aim is to utilize diverse types of wood residues from the forestry industry including *Pinus sylvestris, Pinus pumila, Picea abies, Picea ajanensis, Larix gmelinii, Larix sibirica, Larix sukaczewii, Larix decidua, Abies nephrolepis* (Pinaceae) and *Populus tremula* (Salicaceae) as raw materials, to produce bioactive molecules and environmentally benign technical products as well as bioremediation chemicals. A comparative analysis was carried out of the performance of the MARS microwave extraction system against traditional extraction methods, Soxhlet extraction and shaker extraction. A number of compounds have been isolated and identified from *L. gmelinii, L. sukaczewii, L. sibirica, P. sylvestris, P. pumila and P. abies*. Amongst them the novel labdane diterpenoid 6β,13-dihydroxy-14-oxo-8(17)-labdene from *L. gmelinii*, the novel pumilanoic acid from *P. pumila*, the known and quite unusual serratane triterpenoid, 3β-methoxyserrat-14-en-21-one as well as E and Z bornyl ferulate also from *P. pumila*. Pure compounds are being screened for the following activities: insect antifeedant activity, herbicidal, fungicidal, antiviral and antibacterial, anti-inflammatory and antidiabetic by collaborating institutions.

9. In-vitro Antiplasmodial Activity and Toxicity Assessment of the Ethnobotanical Plant *Cassia fistula*

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Malaria is an infectious disease that affects the poorest populations around the world, with an elevated mortality and morbidity. One of the main factors contributing to the escalating prevalence and distribution of malaria is the emergence and spread of drug-

resistant parasites highlighting a need for the discovery and development of novel, affordable antimalarial treatments. *Cassia fistula* L., (Fabaceae), a semi-wild Indian laburnum (also known as the Golden Shower), is distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil. In Tanzania, Zimbabwe, Mozambique and Brazil, *Cassia fistula* pods are used as a remedy for malaria, blood poisoning, anthrax and dysentery. *C. fistula* fruit was originally screened by Spencer and co-workers during the World War II era in an effort to identify candidates with antimalarial activity. Their results showed that the chloroform extract showed moderate activity when administered orally to *Plasmodium gallinaceum* sporozoite infected chicks. In our follow-up investigation, leaves, fruits and bark of *C. fistula* were extracted with solvents of increasing polarity to generate 4 main fractions (L1-L4). Preliminary screening for *in-vitro* antiplasmodial activity displayed that fractions L2 (chloroform extract) from the leaves exhibited the strongest activity against the chloroquine-sensitive stain *Plasmodium flaciparum* D10 (IC_{50} 12.31 µg/mL). Bioassay directed fractionation was performed by a combination of centrifugal partition chromatography and flash chromatography. Active fractions were subjected to further fractionation until three active compounds were isolated. Using intensive spectroscopic analysis including NMR, mass spectrometry, GC-MS, chemical reactions and with literature, the compounds were identified as: lutein (1), (IC_{50} 5.0 µg/mL), phytol (2) (IC_{50} 5.6 µg/mL) and the most abundant compound in the fraction; di-lineolylgalactopyranosyl-glycerol (DLGG) (IC_{50} 4.5 µg/mL) (3). When the three isolated compounds were tested for their cytotoxicity using Chinese Hamster Ovarian (CHO) cells, only compound 3 showed very weak toxicity (IC_{50} 58.8 µmL)), while the other two compounds were non toxic even at the highest concentration tested. This is the first report for characterization of these compounds from *C. fistula*.

10. Molecular phylogeny of the genus *Massonia*

Andreas Brudermann, University of Graz, Austria

Since the first description of the genus *Massonia* Thunb. ex Houtt. in 1780 about 60 species have been described with this genus name. Presently *Massonia* is regarded to consist of 6 to 11 species according to different authors. In our poster we present the currently most extensive molecular phylogeny of this genus and we discuss the recent species concepts in the light of this phylogeny.

11. Bioactive Tuliposides in Tulip gum

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Certain tulip cultivars produce large amounts of gum in the bulbs when infected with the fungus *Fusarium oxysporium*. Gum production can also be induced by applying the gas ethylene, or lanolin paste containing 2-chloroethyl phosphonic acid (ethepon) or indole-3-acetic acid (IAA) to the bulbs after harvest. Previously, studies on the composition of tulip gum reported it to consist mainly of polysaccharides. The composition of tulip gum has mostly been studied in terms of large macromolecules. The gum's polysaccharides have been analyzed to determine sugar composition and molecular mass. Up to now relatively little is known about the gum in terms of low molecular weight metabolite content. Extracts of tulip bulb gum were analyzed by ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) and were found to contain tuliposides. Tuliposides are glycosides consisting of glucose with one or more α-methylene-γ-butyrolactone side chains. The side chains, when separated from the glucose, form ring structures known as tulipalins. Six different tuliposides and two tulipalins have been reported in various organs of the tulip plant. However, this is the first time they are reported in the gum from tulip bulbs. Gum collected from various tulip cultivars were extracted and analyzed by ^{1}H NMR. All gum samples were found to contain 6-tuliposide B, and in some cases 6-tuliposide A was also present. Isolated tulipalins and tuliposides have previously been tested for various bioactivities, and have been reported to possess antibacterial, antifungal and insecticidal properties. The presence of these bioactive+ molecules in tulip gum may suggest a protective role for this physiological response. Gums produced by other plants (e.g. members of the Hyacinthaceae) in response to biotic factors may also possibly contain interesting bioactive small molecules.

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