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CONTENT

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SL01 Physiological effects of exogenous carbohydrates on gastrointestinal epithelia

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Diseases of the gastrointestinal system are often related with pathological changes of mucous membranes. In an *ex-vivo* system based on porcine colonic tissue various neutral and acidic polysaccharides were tested concerning their bioadhesive potential in order to form artificial mucin layers on colon epithelial membranes. Rhamnogalacturonans with a low degree of esterification and linear oligogalacturonids showed significant bioadhesion against colonic mucous membranes. In contrast highly esterified pectins and neutral polysaccharides were ineffective. Within a structure-activity relationship linear, strongly acidic homogalacturonides were shown to be most adhesive agents. Esterification, branching or non-linear backbone structures will reduce the adhesive properties. The bioadhesive effects were concentration-dependent. Polysaccharide layers, located exclusively on the apical membrane surface of colonic tissue, were visualized by fluorescent microscopy. The adhesion of the exogenous galacturonides on the tissue surface was mediated by interaction with the endogenous mucin, for the release of the endogenous mucines with a mucolytic agent resulted in a decreased bioadhesion of exogenous galacturonides. Additionally, mucin-galacturonide synergism was shown by rheological methods. The artificial mucin layers provide protective effects on colonic mucous membranes against toxic agents as shown by incubation of the tissue with TritonX100.

Tests performed on other gastrointestinal membranes (ileum, gastric membranes, buccal epithelia) showed different behaviour against exogenous polymers: while gastric and buccal membranes could be coated with polysaccharide layers, no such effects were seen using ileum material. The respective differences between the physiological material and the bioadhesive structures are discussed.

Further experiments indicated an other class of chitin-derived oligosaccharides to be strong stimulants of endogenous mucin secretion, leading to an increased mucin-layer on colonic membranes. This effect was shown to be similar with mucin secretion induced by steroids (cortison). The potential positive influence of our oligosaccharides within the treatment of inflammatory diseases is discussed.

SL02 Modified algal and fungal polysaccharides as potential new antiinflammatory drugs

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The adhesion of cells to the endothelium plays an important role in leukocytes recruitment during inflammation. The initial adhesive event is mediated by selectins which bind to oligosaccharide structures. There is large interest to develop inhibitors of this step as new antiinflammatory drugs. However, up to now none of the synthesized molecules proved to be successful. Besides, heparin (H) was shown to interfere with this process. But its high anticoagulant activity is opposed to a therapeutic use in inflammation and its isolation from animal material implicates several disadvantages such as poly-dispersity, contamination risk, shortage of resources. As an alternative approach we are using neutral polysaccharides from algae or fungi as starting material to obtain structurally defined sulfated polysaccharides by chemical modification.

In the presented study, the inhibitory influence on the selectin-mediated cell adhesion of a new class of partial synthetic glucan sulfates (GS) was compared with that of H and structure-activity relationships were established. In adhesion assays, the GS inhibit the L- and P-, but not the E-selectin-mediated cell adhesion. Their activity depends not only on the degree of sulfation and the molecular weight but also on the sulfation pattern of the glucose units. Further, the basic polysaccharide structure was shown to play an important role, e.g. the GS are considerably more active than H.

These results obtained under static conditions correlate well with the effects observed in a flow chamber model. The latter examines the influence of the test compounds on the interactions of selectin expressing cells with a vascular surface imitate containing Sialyl Lewis X under shear flow. H turned out to be inactive in this dynamic test system. However, the GS structure-dependently reduce the number of adhering cells and prolong the rolling velocity of the cells.

In conclusion, the cell adhesion inhibitory potency of GS is suggested to contribute to their in vivo observed anti-inflammatory activity. Since their efficacy-risk ratio is much better than that of H, they may be promising candidates for the development of new anti-inflammatory drugs.

SL03 Isolation of an elemanolide sesquiterpene from *Vernonia anthelmintica* (L.) Willd. seeds traditionally used for psoriasis

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The seeds of *Vernonia anthelmintica* (L.) Willd. (Asteraceae) (VA) have been used traditionally in Indian medicine to treat the skin disease psoriasis (1,2). Several extracts of the seeds of VA were assessed for anti-inflammatory activity using a radioimmunoassay to measure their inhibition on the generation of two pro-inflammatory mediators, thromboxane B_2 (TXB_2) and leukotriene B_4 (LTB_4). A methanol extract of the VA seeds (MET) was identified to display good inhibition of both LTB_4 and TXB_2. Fractionation of this extract was carried out by repeated silica gel column chromatography and preparative reversed phase HPLC. This led to the isolation of vernodalol, a sesquiterpene elemanolide lactone. It was identified from ^1H, ^{13}C and 2D NMR spectra recorded in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard, as well as LCMS. Our studies have enabled for the first time the unambiguous assignment of the 3-carbonyl signals and the six olefinic carbon signals. A fraction of the MET extract composed predominantly of vernodalol as established from LCMS was found to inhibit the generation of both LTB_4 and TXB_2. This indicates the anti-inflammatory effect of vernodalol, a previously unreported effect. In addition, this compound was also found to display antiproliferative effects in an SVK-14 keratinocyte cell line. These anti-inflammatory and antiproliferative results clearly substantiate the traditional use of VA in psoriasis.

Acknowledgements: This research was funded by Phytopharm plc.

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SL04 Quantitative structure-activity relationships (QSAR) of cytotoxic and anti-inflammatory sesquiterpene lactones based on NMR spectral data and GA-PLS statistics

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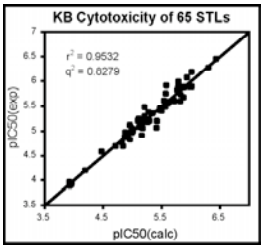
Sesquiterpene lactones (STLs) possess a wide variety of conspicuous biological activities. A major problem concerning their use as therapeutic agents is their high toxicity/low selectivity towards a particular target. A major goal of STL research must therefore be directed towards quantitative structure-activity relationships (QSAR), which might allow disctinction of structural features that render a compound more selective to a wanted biological effect.

In our continuing investigations on QSAR of natural products (1), we introduce here an approach based on the following assumption: if both, activity and molecular spectra, are functions of molecular structure, then it is very likely that activity can be expressed as a function of the molecular spectra (here ^{13}C-NMR spectra). The model-building process was carried out using established methods, i.e. genetic-algorithm-partial least squares regression (2).

NMR data (experimental and simulated) for 65 sesquiterpene lactones were used as spectral descriptors of biological activity with respect to cytotoxicity towards KB cells (see figure). Moreover, a data set of 41 STLs were analysed in the same way for their serotonin release inhibitory activity (3). Finally, semi-quantitative data for 28 STLs' inhibitory effect on NF-κB activity (4) were investigated. The resulting QSAR models are of very high statistical quality, yielding cross-validated correlation coefficients $q^2>0.75$ in all cases, as well as reasonable test set predicitions. It is especially noteworthy, that *calculated* NMR spectra lead to models at least as good as experimental spectra, so that predictions of compounds not available for testing become possible at low computational expense.



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SL05 Kaurane diterpene inhibits NF-κB by targeting DNA-binding activity of p50 and blocks the expression of antiapoptotic and inflammarory NF-κB target genes

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Whole plants of *Isodon japonicus* (Labiatae) have been used in traditional medicine as a remedy for gastrointestinal disorders, cancer, and inflammatory diseases. Despite of its various pharmacological activities, the molecular mechanism of the plant has not been sufficiently explained. We isolated four kaurane diterpene compounds from the plant as an inhibitor of production of inflammatory mediators and NF-κB activation induced by LPS, indicating that these activities of them could explain, in part, *I. japonicus's* diverse pharmacological activities such as anti-cancer and anti-inflammation. We investigated molecular mechanism of a major component kamebakaurin. Kamebakaurin prevented the activation of NF-κB by different stimuli such as LPS, phorbol esters, and TNF-α in various cell types. Treatment of cells with this compound prevent neither the induced degradation of IκB-α nor nuclear translocation of NF-κB by all stimuli. However, this compound significantly inhibited NF-κB activation, and interfered with DNA binding activity of active NF-κB in cell and *in vitro*. Furthermore, kamebakaurin preferentially prevented p50-mediated DNA-binding activity of NF-κB rather than that of RelA as measured using *in vitro* translated p50 and RelA proteins and a p50 mutant with Cys62Ser mutation. These results suggest that this compound exhibit its inhibitory activity by a direct modification of Cys62, which is critical for the DNA-binding activity of p50 subunit. Treatment of cells with kamebakaurin prevented the induced expression of anti-apoptotic NF-κB target genes such as c-IAP1 (hiap-2) and c-IAP2 (hiap-1), and Bfl-1/A1 by TNF-α, resulting in sensitizing MCF-7 cells to TNF-α-induced apoptosis. This compound also inhibited LPS-induced expression of inflammatory NF-κB target genes such as iNOS and COX-2 as well as the production of NO, PGE_2 and TNF-α in RAW264.7 cells, of which may correlated with the result of dose-dependent alleviation of inflammation in a *M. butylicum*-induced adjuvant arthritis model. Based on our results, kamebakaurin could serve as an interesting lead compound for the development of new, potent anti-inflammatory or anticancer agent. Furthermore, this study extends our understanding on the molecular mechanisms underlying the anti-inflammatory and anticancer activities of traditional medicinal plants, which contain kaurane diterpenoids abundantly.

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SL06 Cytokine gene promoter-based *in vivo* screening for identification of novel anti-inflammatory and immunomodulatory herbal compounds/drugs

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In recent years there has been an increasing interest in the use of Chinese herbal medicines for the treatment of inflammatory and autoimmune diseases due to their reputed efficacy. It is desirable to understand the molecular mechanisms by which these medicinal herbs mediate their effects *in vivo*. Cytokines are inducible glycoproteins that play important regulatory roles in the immune system and are often used as therapeutic agents. Here we present a new approach that utilizes cytokine promoter-driven luciferase reporter-gene expression as a target for screening novel herbal drugs and evaluating their underlying molecular mechanisms *in vivo*. The promoter regions of important human pro-inflammatory cytokines such as TNF-α and GM-CSF were isolated, cloned into pGL-3 vector and the resultant plasmids were transfected into mouse epidermal tissues using a particle-mediated gene gun. The blasted skin was treated with crude extracts, solitary test herbal compounds or inflammatory agents. The naphthoquinones from *Lithospermum erythrorhizon* Sieb. & Zucc., in crude extract as well as the pure individual compounds shikonin, isobutyryl shikonin, acetyl shikonin, dimethylacrylic shikonin and isovaleryl shikonin showed significant dose dependent inhibition of TNF-α promoter activity induced by gene gun. The commercially available topical anti-inflammatory steroids hydrocortisone and betamethasone were also found to inhibit TNF-α promoter activity in our system. Croton oil, a well-known skin inflammation inducer, readily increased the transgenic GM-CSF-Lux promoter activity by 7-fold over the original control, whereas shikonin effectively decreased the GM-CSF promoter activity to 10-fold less than that of the inflamed controls. This investigation provides an *in vivo* system to understand the possible molecular basis for the therapeutic properties of traditional herbal medicines and also a molecular screening method to identify the novel therapeutic and/or immune modulatory agents for anti-inflammation and topical immunotherapy.

SL07 Phytoequivalence of botanical derivatives: new perspectives

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The possibility to standardize extracts is nowadays a feasible task due to the combination of strict conditions of plants cultivation and/or harvesting (GAP) and rigorous industrial procedures for the extraction until the final product (GMP).
By using this approach it is now possible to prepare very well characterized and reproducible extracts that can be submitted to rigorous preclinical and clinical investigations according to pharmaceutical guidelines of western countries. In several countries, such as USA, UK, Canada, Italy and others, standardized extracts are also commercialized in non-pharmaceutical channels and they are commonly considered "dietary supplements" or "nutraceuticals".
Differently from the pharmaceutical market, where proprietary rights are considered a pivotal aspects and consequently protected by specific rules, in these parallel fields, the correspondence between the documentation and the composition of the products on the shelf is not a strict issue. This aspect is crucial causing a double damage respectively to the consumers which are not guaranteed for safety and efficacy and to the producers of standardized and well-documented products which have serious disadvantages in term of protection.
In this perspective, the problem of "phytoequivalence" is becoming more and more a strategic issue. Nowadays, the utilization of sophisticated analytical techniques allows to recognize differences in composition of extracts not only related to specific components but also to the unknown part.
The combination of specific HPLC analysis with semiquantitative ^{1}H- and/or ^{13}C-NMR or NIR (Near Infra Red) Spectroscopy represents a good approach to answer to the problem of phytoequivalence.
According to this combination of techniques, several products have been compared for their equivalence; in the specific instances of *Ginkgo biloba, Hypericum perforatum* and grape seeds standardized extracts prepared by different producers, it was possible to verify striking differences in term of composition profiles.

SL08 Transaminase and alkaline phosphatase activity in the serum of burn patients treated with highly purified tannic acid

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The use of tannic acid in the treatment of burns has a long and successful history (1). In the early nineties, preliminary experimental and clinical studies confirmed these positive reports from the past and indicated that highly purified tannic acid (HPTA) might be of interest as a valuable additional therapeutic regimen to improve long-term wound-healing characterisitics after thermal injury (2,3). However, prior to introduction of HPTA on a broader scale, previous publications on alleged hepatotoxic effects (1) must be negated. This necessitates further establishment of the effects of HPTA on the liver, as well as to prove its general non-toxicity. As a first step in such a safety evaluation, we report here on the results of a retrospective study into the serum transaminase and alkaline phosphatase activity of burn patients already treated with HPTA (3). Temporary elevations in the activity of gamma-glutamyl transferase, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were observed in both HPTA-treated patients and their matched controls. No statistically significant difference (Student's *t*-test and multiple linear regression) was found between the two patient groups with respect to the mean enzyme activities, calculated as the areas under the curve between five and 15 days post-burn. These results seem to indicate that HPTA is not hepatotoxic, at least when applied to a burned area corresponding to approximately ten percent of the total body surface. This is in agreement with the widespread and frequent use of HPTA in the food, cosmetic and pharmaceutical industries.

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SL09 Red wine polyphenols promote endothelial nitric oxide release by enhancing endothelial nitric oxide synthase expression

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Population based studies suggest a reduced incidence of morbidity and mortality from coronary heart disease by moderate and regular consumption of red wine (1). Endothelial nitric oxide (NO) is a pivotal vasoprotective molecule. In addition to its vasodilating feature, endothelial NO has anti-atherosclerotic properties by inhibiting platelet aggregation, leukocyte adhesion, smooth muscle cell proliferation and the expression of genes involved in atherogenesis (2). This study, therefore, examines the influence of red wine polyphenols on the regulation of eNOS expression and subsequent NO synthesis focusing at putative long-lasting anti-atherosclerotic effects of red wine. Treatment (20 h) of human umbilical vein endothelial cells (HUVECs) and of the HUVEC-derived cell line EA.hy926 with a alcohol-free red wine polyphenol extract (RWPE) led to a dose-dependent (100-600 µg/mL), significant increase in NO release (up to 3.0-fold/HUVEC and 2.0-fold/EA.hy926) by use of the fluorescent probe DAF-2 (4,5-diaminofluorescein). This effect was corroborated by the [^{14}C]L-arginine/L-citrulline conversion assay in intact EA.hy926 cells. RWPE (20 h, 100-600 µg/mL) also significantly increased eNOS protein levels up to 1.8-fold. Furthermore, we found an increased human eNOS promotor activity (up to 1.9-fold) in response to red wine polyphenols (18 h, 100-600 µg/mL) as demonstrated by a human eNOS-luciferase reporter gene assay. We provide conclusive data showing for the first time that a RWPE increases eNOS expression and subsequent endothelial NO release. Increased active eNOS levels may antagonize the development of endothelial dysfunction and atherosclerosis supporting the view that red wine indeed may have long-term protective cardiovascular properties mediated by its polyphenols.

Acknowledgements: We thank Dr. Véronique Cheynier, INRA-UMR Sciences pour l'Oenologie, Montpellier for providing chemically characterized red wine polyphenol extract.

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SL10 Selective estrogenic activity of *Vitex agnus-castus*

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Extracts of the fruits of Vitex *agnus-castus* (VAC) are commonly used for the treatment of premenstrual symptoms, corpus luteum insufficiency and menstrual cycle length disorders. In our screening programme for plant extracts with estrogen-like activity we also included VAC. In a receptor-binding assay performed with recombinant human ER the VAC extract BNO 1095 showed a preferential binding to ERβ over ERα, thus revealing the quality of a phyto-SERM.

The extract was fractionated at Sephadex LH20 as stationary phase with 75% (v/v) ethanol as mobile phase. Column dimensions were 500 cm x 5 cm, flow rate was adjusted with a pump to 1 mL/min. Aliquots of the effluent were tested for their binding strength to a cytosolic preparation from porcine uteri. Active fractions from this assay were collected and submitted for differentiation reasons to a second binding assay to human recombinant ERα or ERβ. The compound did not bind to the ERα but had clear affinity towards the ERβ.

Elucidation of the structure of the active compound was achieved by TLC, comparison of retention times in HPLC, UV-VIS-spectroscopy, ^{1}H- and ^{13}C-NMR. The physico-chemical data of the isolated compound and apigenin used as reference compound were identical.

Apigenin has been described to possess preferential binding activity to ERβ over ERα by Kuiper *et al.* but to our knowledge the compound was not yet described to occur in VAC, even if the occurrence of vitexin and isovitexin in the plant make the presence of the aglycone plausible.

References: Kuiper, GG. et al. (1997), Endocrinology, 138: 863-870.

SL11 Determination of estrogenic activity with use of an ER reporter gene system

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Phytoestrogens, widely distributed in the plant kingdom, are currently receiving considerable attention as a potential alternative therapy for a range of hormone-dependent conditions including post menopausal symptoms, prevention of breast and prostate cancer, and protection against coronary heart disease and osteoporosis. The existence of two receptor subtypes ERα and ERβ with both their own tissue distribution and biological characteristics, makes it of great importance to determine the receptor-specific activity of phytoestrogens. In our institute we make use of 293 human embryonal kidney cells stably transfected with either ERα or ERβ combined with a luciferase response element (reporter gene). In this system we are able to detect (our standard) 17-β estradiol at concentrations as low as 10^{-14} M; maximum responses are detected at concentrations of 10^{-11} M. The maximum response is taken as 100% and phytoestrogenic activities of several well-known compounds are expressed as percentage of maximum response at a certain concentration; see table below:

	ER-α		ER-β	
compound	response	conc.	response	conc.
17β-estradiol	100	10^{-11} M	100	10^{-11} M
Genistein	55	10^{-7} M	151	10^{-7} M
Daidzein	100	10^{-6} M	155	10^{-6} M
8-Prenylnaringenin	98	10^{-9} M	180	10^{-9} M
Coumestrol	85	10^{-8} M	150	10^{-8} M

We conclude that the estrogenic potency of phytoestrogens is significant, in particular concerning ERβ. With the ER reporter gene system we posses an elegant and efficient tool for screening of phytoestrogens in the plant kingdom and evaluation of herbal extracts.

SL12 Leucamide A: a new cytotoxic heptapeptide from the Australian sponge *Leucetta microraphis*

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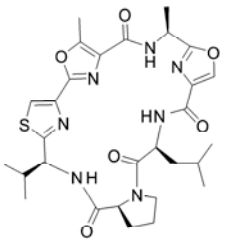
Leucamide A (**1**), a bioactive cyclic heptapeptide containing a unique mixed 4, 2-bisheterocycle tandem pair consisting of a methyloxazole and thiazole subunit was isolated using RP HPLC together with the known compound BRS1 (**2**), from the dichloromethane extract of the Australian marine sponge *Leucetta microraphis*. The planar structure of leucamide A (1) was elucidated by employing spectroscopic techniques (NMR, MS, UV, and IR). Its absolute stereochemistry was established by chemical degradation, derivatisation and chiral GC-MS analysis. A conformational analysis of 1 was made using MMFF. Leucamide A (1) was found to be moderately cytotoxic towards liver and stomach tumour cell lines.



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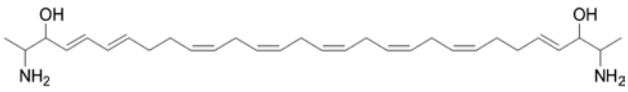

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SL13 New trends in the biochemistry and pharmacology of methoxylated lipids

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The biochemistry and pharmacology of methoxylated lipids continues to attract much attention, in particular that of alkylglycerol ethers and fatty acids bearing the methoxy group in the alkyl chain. An interesting group of methoxylated lipids are the α-methoxylated fatty acids since the only naturally occurring α-methoxy fatty acids known to date are those derived from the phospholipids of sponges (1-4). We examined the lipid composition of a series of Caribbean sponges (*Callyspongia fallax, Amphimedon complanata, Agelas dispar*) and were successful in identifying a novel series of 2-methoxylated fatty acids ranging in chain-length between C_{14} and C_{18} (2-4). These fatty acids included linear chain saturated 2-methoxylated fatty acids between C_{14}-C_{18}, a series of novel *iso-anteiso* branched-chain 2-methoxylated acids with chain lengths between C_{15}-C_{17}, and also an unprecedented series of Δ6 normal chain 2-methoxylated fatty acids with chainlengths between C_{14} and C_{18}. Structure characterization was accomplished by means of gas chromatography retention times, gas chromatography-mass spectrometry, and total synthesis. These findings revealed unprecedented fatty acid biosynthetic sequences in nature. The antiviral activity (against HIV-1) of the 2-methoxytetradecanoic acid, the shortest α-methoxylated fatty acid known to date, will be discussed as well as its potential as a *N*-myristoyltransferase inhibitor. In addition, some of the a-methoxylated fatty acids show selective antimicrobial activity against Gram-positive bacteria (MIC = 0.35 μmol/ml). Our present knowledge in this field, in particular the natural occurrence, biological activity, and synthesis of this interesting group of lipids, will be discussed.

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SL14 Pentameric ellagitannins from *Monochaetum multiflorum* (Bompl.) Naudin, *Melastomataceae*

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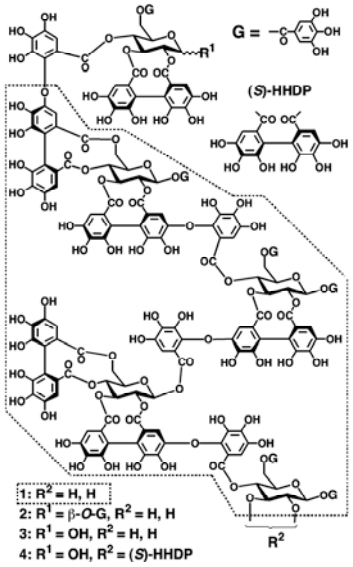
Monochaetum multiflorum (Bompl.) Naudin, a shrub endemic to Colombia, has been traditionally used as a topically applicable remedy against infections and skin injuries. A previous paper reported the isolation of nobotanin S (**1**) (tetramer), and the first pentameric hydrolyzable tannin, named melastoflorin A (**2**) (1).

This communication describes the isolation and structure elucidation of two new pentameric ellagitannins, melastoflorin B (**3**), $[\alpha]_D$ +62.9˚, $C_{184}H_{128}O_{118}$, and melastoflorin C (**4**), $[\alpha]_D$ +60.0˚, $C_{198}H_{134}O_{126}$. Although the glucose proton signals in the ^{1}H-NMR spectrum were complicated owing to overlapping around 5~5.2 ppm region, full assignments were unambiguously achieved by a combination of ^{1}H-^{1}H shift correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), ^{1}H-^{1}H *J*-resolved, GHMQC and HMBC spectra.



Acknowledgements: Ministry of Education, Science, Sport and Culture of Japan.

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SL15 New antiproliferative kaurane-type diterpenes from *Parinari sprucei*

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In the course of our investigations on Latin American medicinal and food plants, we have been studing many Venezuelan species belonging to the Chrysobalanaceae with the aim of isolating as many secondary metabolites as possible, for better phytochemical and chemotaxonomic characterization of the family, and for subjecting the isolated compounds to biological screening on the basis of their structural relationships with similar metabolites or known drugs (1, 2).
In this context we selected *Parinari sprucei* Hook. f., a tree up to 20 m in height growing in the Amazon forest of Venezuela, whose fruits are edible and constitute part of the diet of the Indios living in the region of Cataniapo river, where the plant was collected (3). The genus *Parinari* is phytochemically not deeply investigated: only few species were studied leading to the identification of some nor-kaurene and *ent*-kaurene diterpenes (4, 5). In this work we report the isolation, by modern chromatographic methods (Sephadex LH-20 and SPE column, HPLC), and structural characterization of twelve new kaurane-type diterpenes from the leaves of *P. sprucei* by means of high resolution 1D- and 2D-NMR (COSY-DQF, TOCSY, HSQC, HMBC, ROESY, and NOESY) experiments, as well as by ESI-MS analysis. Since some kaurane diterpenes exhibited cytotoxic activity (6, 7) pure compounds obtained were tested for evaluating their antiproliferative activity using three continuous cell lines: J774.A1, WEHI-164, and HEK-293. The cell viability was assessed through an MTT conversion assay (8). Some diterpenes showed good activity in comparison with 6-mercaptopurine used as reference compound.

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SL16 Significance of medicinal plants in Northeast Brazilian health care – Farmácia Viva

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Health care organisation in Brazil reflects the social structure of the country. High technology private care is available to the rich whereas the vast majority must make do with inadequate public care. Financial resources are concentrated on the hospital sector of the few big cities and only 15% of public health funds are left for primary health care. The majority of the population depends on self-medication. Medicinal plants are used in Brazil traditionally and there is widespread knowledge of therapeutically relevant species, knowledge which is in danger of being lost or mixed with perverted versions of popular medicine currently in fashion. A confusing number of simultaneous plant names and the vast variety of adulterants and substitutes lead to high risk of erroneous application of certain species. These risks are demonstrated by three examples of often used local plants: *Plectranthus barbatus* Andr., *Cymbopogon citratus* Stapf. and *Mentha x villosa* Huds. In Northeast Brazil the comprehensive and integrated pharmaceutical-social program "Farmácia Viva", ('living pharmacy') was created to help underprivileged people correctly and effectively use those plants whose medicinal properties have been validated through scientific study. The intention is to support communities in preparing their own natural medicine for primary health care under technical supervision. Selected local plants are analysed for their active principles and therapeutic relevance and plant monographs are elaborated. Those plants which meet the specific needs of a distinct area are selected. Health professionals and laypersons in the communities are oriented and supervised in the cultivation and harvest of plants as well as the preparation and application of the phytotherapeutics. Direct contact and exchange with local people provides additional empirical data to encourage new scientific studies. The "Farmácia Viva" project is presented as an exemplary program to deal with the lack of governmental primary health care in Northeast Brazil and to link scientific pharmaceutical expertise with traditional and popular knowledge of medicinally relevant plants as a source for further scientific studies.

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SL17 BioArena: hyphenation of OPLC with bioautographic detection

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Recently, the resistance of microbial strains to antibiotics is a big problem in the field of anti-microbial animal and human therapy. Microbes have developed mechanisms of resistance to all classes of antibiotics available for systemic use in humans. Therefore, the research of these resistance mechanisms and the search for new antibiotics or antibiotic-like substances are actual tasks of the pharmaceutical science. The biological systems as microbes or plants contain thousands of constituents and are a valuable source of new and biologically active molecules, e.g. antibiotics or antibiotic-like substances. For their investigation, it is important to have suitable biological assays and chemical screening methods. Among the bioassays, the direct bioautography is applicable to microorganisms that can grow directly on a chromatoplate after the separation (1). There is a possibility for an advantageous combination of the layer liquid chromatography with the direct bioautography, so all the steps of the combined method (separation of the constituents, pre-conditioning, incubation, visualisation) are performed on the same sorbent layer. It is obvious that a column system is not suitable for such investigations. Among the layer liquid chromatographic techniques, over-pressured layer chromatography or optimum performance laminar chromatography (in short form: OPLC) integrates the advantages of the conventional TLC/HPTLC and HPLC. The combination of bioautography with the automated OPLC results in the so-called BioArena (2) as a complex bioautography system which exploits attractively the advantages of OPLC giving compact spots and good resolution and sensitivity. This system provided optimum conditions for the detection of ingredients from grapes, cabbage and paprika as unique medicinal plants. However, BioArena generates also other advantageous features. It can be used for studying the role of changeable incubation time in the mechanism of action of antibiotics and the interactions between the microbes and the dye substance as well as other small and big molecules as co-factors in the sorbent bed after OPLC separation.

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SL18 Biosensoric detection of the cysteine sulphoxide alliin

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Garlic (*Allium sativum* L.) and related species of the Alliaceae family are known for their cancer-protecting and antiatherosclerotic potential. Sulphur containing flavour compounds are responsible for the characteristic smell and taste of members of this family. These volatile flavour substances are formed by the action of alliinase (EC 4.4.1.4) on cysteine derivatives, when plant material is disrupted (1). Intact bulbs contain mainly the odourless, nonvolatile precursors such as (+)-S-(2-propenyl)-L-cysteine sulphoxide (alliin).

In the present investigation, an alliin-specific biosensor exploiting immobilized alliinase has been developed. Besides volatile compounds like allicin, also pH-active substances as pyruvic acid and ammonia were formed, which can be detected by a pH sensitive electrode (2, 3). Enzymically formed ammonia was detected either by a potentiometric sensor based on an ammonia electrode or a pH-sensitive electrolyte / insulator / semiconductor (EIS) layer structure made of Al/p-Si/SiO_2/Si_3N_4. It could be demonstrated with both methods that this biosensoric method yielded results comparable to sensitivities obtained by HPLC. Alliin concentrations down to $6x10^{-6}$ M could be quantified.

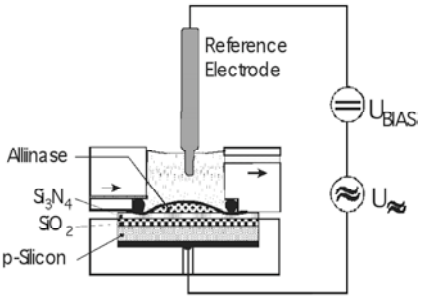

Figure Experimental set-up of the alliin biosensor (EIS). The cavity above the alliinase-layer contains the sample solution.

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SL19 *Agrostemma githago* L. Isolation of the toxic compounds and new approaches to their mode of action

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In the course of our investigation of *Agrostemma githago* L. var. *githago*, a well known toxic member of the Caryophyllaceae family, to date three triterpenoid saponins have been isolated with gypsogenin (3β-hydroxy-olean-12-en-23-al-28-oic acid) as aglycone (1). A combination of these particular saponin derivatives with a formyl function in triterpene position 4 (3 µg/ml) together with agrostin, a glycoprotein (M_r: 27 kDa), showed comparable toxicity against an endothelial ECV-304 cell line. In order to reproduce our results, we isolated Agrostin (3), a ribosome-inactivating protein (RIP type 1) from the seed of *A. githago*. After extraction, filtration, centrifugation and $(NH_4)_2SO_4$-precipitation, crude extracts were dialysed against 5 mM sodium-phosphate buffer (pH 6,5) and applied to a Sephadex column (Sephadex G 50/75) and to a CM-cellulose column. M_r values were determined by polyacrylamide-gel-electrophoresis and quantification of the isolated protein was determined by the Bradford- and the BCA-protein assay. Subsequent antigen-antibody-testing was undertaken for identification and quantification of the protein. Fluorescent microscopy imaging is used for intracellular detection of stained Agrostin. In order to obtain both active compounds from the seed material, we isolated an active Agrostemma-saponin from the seeds of *A. githago* (2). Repetition of our in vitro experiments with both isolated substances revealed the expected toxicity. No analogy could be drawn between the observed induction of RIP-toxicity of Agrostin and the induction of apoptosis by FAS-C-terminal tripeptide through Agrostemma-saponin, suggesting that these peptides use a different mechanism to penetrate through the cell membrane.

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SL20 Cyclotides - plant defense peptides with anticancer lead potential

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Several members of the Violaceae and the Rubiaceae plant families produce peptides of about 30 amino acids with a remarkable 3-dimensional structure, including a head-to-tail cyclised backbone and three disulfide bonds arranged as a cysteine knot. These peptides, referred to as cyclotides (1), have a potential role in the plant host defense system (2).

We have developed specific methods for isolation and structure elucidation of cyclotides. A fractionation protocol is used for the isolation of highly purified cyclotide fractions and for the removal of substance classes know to interfere with bioassays e.g., tannins (3-4). Examples of the methods used for structure elucidation, i.e. mass spectrometry sequencing and homology modelling, are presented in this poster.

In addition, we show that cyclotides from *Viola* sp. have cytotoxic activity in human cell lines using a fluorometric microculture cytotoxic assay (FMCA) (5). Activity profile of cyclotides differs significantly from those of anticancer drugs in clinical use today, indicating a new mode of action (6). The dose response curves show a very sharp profile, a phenomenon also described for a similar host defense peptide family, called defensins (7). A likely mode of action, formation of pores in the cell membranes, is discussed.

The spectacular biological and chemical stable structure of the cyclotides and a possible new mode of cytotoxic action, represent an interesting starting point in the design of new anticancer leads.

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SL21 Characterisation of St. John's wort extracts by multivariate analysis of spectroscopic data

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Herbal medicines, produced from plant materials, often present a unique problem for manufacturers desiring the characterisation, reproducibility and standardisation that are required of pharmaceuticals. This problem is primarily due to the plurality of components contained in a herbal medicine and the large variation in composition. For the majority of plant extracts there is no evident correlation to be found between pharmacological activity and certain characteristic compounds. Although it is evident that the whole extract must be considered as the therapeutic agent, the characterisation is carried out referring to one single -often inactive- compound. Accordingly, there is a need to provide alternative methods for standardising complex botanical materials.
In our present work we choose a new approach for the classification of thirty different extracts of *Hypericum perforatum* adopting a method that has been developed in studies of "metabolic profiling" (1). We show the application of proton NMR spectroscopy as a very general analytical chemical tool for the characterisation of crude plant extracts. This technique can quantitatively and simultaneously detect all proton-bearing compounds and consequently all relevant substance classes in the samples. However, the spectra obtained are too complicated to be analysed visually. Therefore, the classification of spectra in this study was carried out using several multivariate statistical methods: Principal component and discriminant analysis as well as nonlinear regression techniques were used for the visualisation of the complex data set. In order to correlate the spectral data with pharmacological information, we describe the calibration of a quantitative model using a PLS algorithm. We also show that principal component loading plots and factor spectra are an effective tool in the interpretation of the differences between the substance composition of each extract.

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SL22 Ability of hederacolchiside A_1 to bind melanin may partly explain its strong antiproliferative activity on human melanoma cells

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It was demonstrated that hederacolchiside A_1 (Hcol-A_1), a monodesmoside from *Hedera colchica* K. Koch, with the sugar sequence O-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside at C3 of oleanolic acid and a complementary glucopyranosyl moiety branched at C1 of arabinose, exhibits *in vitro* stronger anti-proliferative effects in human malignant melanoma cell-line M4 Beu (IC_{50}: ca 4.5 µM) than in a panel of carcinoma cells, with differential cytotoxicity versus normal fibroblasts (IC_{50}: ca 7.5 µM) (1). The present study focused on mechanisms involved in the stronger activity on melanoma cells. Complementary investigations on four melanoma cell-lines, showed the weakest activity on human melanoma M3Dau, a cell-line which do not express melanin, suggesting that the anti-melanoma activity of Hcol-A_1, might be partly related to a specific ability to bind melanin. This hypothesis was verified by *in vitro* experiments with a new NMR technique, high resolution magic angle spinning and, insoluble synthetic melanin. ^1H spectra of Hcol-A_1 in D_20 phosphate buffer pH 7.4 were recorded at 500 MHz with a Bruker AVANCE DRX spectrometer fitted with a HRMAS probe, in presence and in absence of melanin. Interaction with melanin was demonstrated by a concentration-dependent linear broadening of line-widths which allows to determine the equilibrium dissociation constant (Kd) saponin-melanin.

Acknowledgements: INTAS program (Project 01-2043).

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SL23 A subcutaneous microdialysis method combined with ESI LC–MS analysis for studying the skin penetration of tryptanthrin in Isatis extracts

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Isatis tinctoria L. (woad, family Brassicaceae) is an old European and Chinese dye and medicinal plant with a well documented history as an anti-inflammatory. Oral as well as topical application has been described in the ancient herbals. The anti-inflammatory potential of woad was recently confirmed in a broad pharmacological screening and the alkaloid tryptanthrin identified as an active principle with potent inhibitory properties on COX-2 and 5-LOX catalyzed eicosanoid synthesis (1, 2). Topical application of *Isatis* extracts in the TPA-induced ear oedema model in mice recently confirmed a significant anti-inflammatory effect *in vivo*. In view of a clinical study of *Isatis* extracts in topical application, analytical tools were needed for a suitable monitoring of the skin penetration of active principles in woad extracts.

Skin microdialysis allows for a time-resolved determination of local drug concentrations in volunteers (3). We established and validated a method for tryptanthrin using pig foreleg as a model. Microdialysis was carried out with a hollow fibre (i.d. 200 µm, exclusion limit 5000 amu) placed in the dermis at 1 to 1.5 mm below the skin surface. The flow rate of the dialysis fluid was 2 µl/min. Defined solutions of tryptanthrin and woad-extracts were applied onto the skin area above the fibre. Tryptanthrin concentrations in the dialysate were determined by ESI LC-MS, using d_8-tryptanthrin (4) as internal standard. A short, narrow-bore HPLC column (Purospher C-18 endcapped, 3 µm, 55 x 2 mm i.d.) was used without eluent split and with detection in the SIM mode (LOD: 100 pg; LOQ: 500 pg). In the pig forleg model, measurable tryptanthrin concentrations were found in the dialysate already 20 min after topical application of test compound or extract. Curves were recorded for 4,5 h. Depth of the fibre and amount of tryptanthrin affected the concentrations in the dialysate. Tryptanthrin penetration from extracts was proportionally higher than when a solution of pure compound was applied. Other extract substances may thus enhance the penetration of the poorly soluble alkaloid.

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SL24 Development of analytical methods for the biosafety assessment of genetically modified organisms

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Due to the lack of scientific knowledge, the use of genetically modified organisms (GMO) in the food industry is a major subject of controversy. Very recently, the concept of substantial equivalence of antinutrients was given (1) to regulate the introduction of novel food to the European market opening the door to a new analytical challenge: how to evaluate the biosafety of genetically modified organisms? In this context, the development of new techniques and methods of analysis for the collection of further comparative data through fingerprinting (metabolome) and quantitative determination of plant secondary metabolites is of main concern in order to determine whether GMO constitute a risk to human health or the environment. Choosing the potato tuber as a model of study, different genetically modified Bintje commercial potato variety were grown both in greenhouse and in the field in order the increase resistance to late blight (*Phytophtora infestans*). A HPLC/UV method was developed to quantify α-solanine and α-chaconine, the two major alkaloids found in potato tubers and well known for their toxicity. A preliminary screening obtained with greenhouse grown potato tubers indicated significant quantitative variations of both α-solanine and α-chaconine between the original Bintje potato and the genetically modified ones. In order to assess such results in a natural environment, genetically modified and unmodified Bintje potato variety were grown in the field together with other commercial potato varieties. A LC/DAD-UV/MS method was developed using the dichloromethane potato extract in order to obtain a fingerprint of the lipophilic constituents. Comparison of the fingerprints of genetically modified and non modified potatoes showed significant quantitative differences of some metabolites. Identification of these metabolites as well as quantification of α-solanine and α-chaconine in these potatoes is on course.

Acknowledgements: The authors would like to thank the Office Fédéral de l'Environnement, des forêts et du paysage of Switzerland for financially supporting this project.

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SL25 An *in vitro*: *in vivo* fusion system for optimised production of St. John's wort (*Hypericum perforatum* L.)

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The efficient production of St. John's wort (*Hypericum perforatum* L.) requires a fusion of growing systems in controlled environments to ensure that the biochemical profile of the resulting plant material has the highest possible quality. Wild harvested and cultivated St. John's wort has a broad diversity of chemotypes arising from spontaneous apomixis in seed development, pollenation, and environmental effects resulting in variable synthesis and accumulation of specific compounds. Therefore, an *in vitro* system for clonal propagation via cytokinin-induced *de novo* shoot organogenesis was developed to provide sterile, uniform plant material for investigations. Exposure of etiolated hypocotyls or sterile stem segments to a medium containing 5 µM thidiazuron (TDZ: N-phenyl-N'-(1,2,3-thidiazol-yl)-urea) for 6-9 days with subsequent transfer to a medium devoid of growth regulators resulted in the development of 25-40 shoots per explant. The regeneration protocol was used to generate a series of selected lines originating from a single seed. *In vitro* propagated plantlets of line SJW17 were transferred to a controlled environment greenhouse, acclimatized to a hydroponic system and grown to maturity for tissue collection. Flowers were harvested from 2-month-old plants and subjected to biochemical analyses. Hypericin, hyperforin and pseudohypericin were present in the flowers at comparable concentrations to previous reports for field-produced plant materials. Melatonin and serotonin, indoleamine neurohormones associated with circadian rhythms and anti-oxidation pathways, were quantified in the *in vitro* plantlets and the *in vivo* flower tissues. Melatonin was quantified in leaf (1.8 µg/g), flower (4.4 µg/g), stem (1.9 µg/g), and etiolated hypocotyls (59.8 µg/g). Radiolabel from ^{14}C-tryptophan was recovered as ^{14}C-melatonin in sterile plantlets indicating endogenous synthesis of the compound in St. John's wort. Analysis of the flower buds at six different stages revealed that serotonin was present during the tetrad stage of anther development while melatonin was detected at high levels during uninucleate mircospore development. Together, these investigations have demonstrated that a fusion of *in vitro* and *in vivo* systems can be effectively used for efficient production and discovery of novel compounds in St. John's wort and other medicinal plants.

SL26 Synthesis, cytotoxicity and antiplasmodial activity of new *indoloquinoline* derivatives

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Based on the original lead neocryptolepine or 5-methyl-5*H*-indolo[2,3-*b*]quinoline, an alkaloid from *Cryptolepis sanguinolenta*, a series of derivatives was prepared using a biradical cyclisation methodology. Starting from easily accessible products, this approach allowed the synthesis of hitherto unknown compounds with a varied substitution pattern. As a result of steric hindrance, preferential formation of the 3-substituted isomers over the 1-substituted isomers was observed when cyclising *N*(3-substituted-phenyl)-*N'*-[2-(2-trimethylsilylethynyl)phenyl]carbodiimides.

All compounds were evaluated for their activity against chloroquine-sensitive as well as chloroquine-resistant *Plasmodium falciparum* strains, and for their cytotoxicity on human MRC-5 cells. Mechanisms of action were investigated by testing inhibition of β-haematin formation, and DNA interactions (DNA-methylgreen assay).

Neocryptolepine derivatives with a higher antiplasmodial activity and a lower cytotoxicity than the original lead have been obtained. This selective antiplasmodial activity was associated with inhibition of β-haematin formation. 2-Bromoneocryptolepine was the most selective compound with an IC_{50} value against chloroquine-resistant *P. falciparum* of 4.0 µM in the absence of cytotoxicity (IC_{50} > 32 µM). Although cryptolepine, a known lead for antimalarials also originally isolated from *Cryptolepis sanguinolenta*, was more active (IC_{50} 2.0 µM), 2-bromoneocryptolepine showed a low affinity for DNA, in contrast to cryptolepine.

Although some neocryptolepine derivatives with a higher antiplasmodial activity than 2-bromoneocryptolepine were obtained, these compounds also showed a higher affinity for DNA and/or a more pronounced cytotoxicity. Therefore, 2-bromo-neocryptolepine is considered as the most promising lead from the present work for new antimalarial agents.