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ABSTRACTS

IMPROVEMENT OF RECOMBINANT *PICHIA PASTORIS* EXPRESSION SYSTEM FOR INDUSTRIAL APPLICATION

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We report our partial results of a long-term project for the industrial application of recombinant *Pichia pastoris* strains. The project covers the transformation of target protein genes into the host strain, screening for expression, optimization, kinetic modelling and scale up of fermentations as well as the downstream processes of the recombinant products. The project involves technical improvements connected to the establishment of successful expression systems, as well.

The product formation of *Pichia pastoris* Mut^+ is induced and determined by the methanol concentration in the fermentation broth. Two ways of the control of methanol concentration are generally accepted: direct measurement of methanol or dissolved oxygen controlled substrate feed [1]. First a self-developed, organic-sensor based methanol concentration measurement system was examined. The parameters, which control the output signal of the sensor were determined in second order statistical experiments both for water-methanol and fermentation-broth – methanol systems. A suitable model was set up which can be applied for sensor design, as well as for control purposes. In order to test capabilities of dissolved oxygen controlled substrate feed, Zhang's kinetic model was completed with an oxygen balance equation based on metabolic considerations [2]. The model parameters were fitted to experimental data but the results proved not to be adequate due to putative physico-chemical changes caused by the methanol concentration changes in the system, which were not involved in the model. Experimental data were also evaluated statistically. The results of the statistical analysis imply that

through the mixture of effects on the dissolved oxygen concentration, no adequate control strategy can be set up based on dissolved oxygen concentration.

In order to make comparative screening for the expression of intracellularly expressed recombinant proteins, batch and continuous laboratory-scaled ultrasonic disintegrations were optimised. For scale up purposes, high-pressure and glass-bead homogenisations were compared with laboratory scale methods. After optimisation we found that, both laboratory scale ultrasonic methods and high-pressure homogenisation could reach total cell disruption.

Finally, bacterial 1,3-propanediol-oxydoreductase producing *Pichia* strains were tested in previously optimised spinning test tube and shake flask experiments. The best producing strain was cultivated in bench-top fermentors with the methanol-sensor-based substrate inlet control. Cells were disrupted by the optimised ultrasonic disintegration method. The recombinant bacterial enzyme expressed with His_6 tag had full activity and could be purified by the combination of size-exclusion and Ni-affin chromatography. The enzyme activity measured from the crude *Pichia* cell-extract was three-times higher than in the case of the original *Enterobacter* strain. The enzyme was not stable at room temperature or at $4\,^{\circ}C$ but could be stored without significant decrease of activity at $-20\,^{\circ}C$ for 2 months.

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MONOLITHS SYNTHESIZED BY RADIATION POLYMERIZATION

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Porous polymer blocks called monoliths are attractive alternatives over conventional packed bead columns for fast separation of proteins and peptides, because the mass transfer within the monolith is accelerated by convection. Besides in chromatography, monoliths are useful in many different applications, such as catalysis, separation, diagnostics and peptide synthesis.

Radiation initiated polymerization and crosslinking was used for the synthesis of poly(diethyleneglycole dimethacrylate), poly(hydroxyethylacrylate-*co*- diethyleneglycole dimethacrylate) and poly(glycidyl methacrylate-*co*-diethyleneglycole dimethacrylate) monoliths. This method permits the preparation of monoliths of any desired size and shape in situ, eliminating thus the column filling procedures. The ease of the preparation, the short reaction time and the possibility of running the reaction at any temperature are some of the advantages of radiation over thermal initiation of polymerization. The pore formation is controlled by solvent quality, monomer concentration, irradiation dose, dose-rate and temperature. Teflon tubes were filled with solutions containing different monomer concentrations up to 55 w/w % in various solvents (methanol, ethanol, propanol, butanol, acetone, etilacethate, tetrahydrofurane, acetonitrile and dioxane) and irradiated in nitrogen atmosphere with doses up to 50 kGy. The dose rates used were in the range of 1–15 kGy/h, and the irradiation temperature was changed from 0 °C to 60 °C. After irradiation the tubes were provided with fittings and attached to chromatographic pump to measure the flux at different pressures. The characterization of monoliths was performed by electron microscopy and mechanical testing. The pore size and distribution was determined by mercury intrusion porosimetry, and specific surface area was calculated from the adsorption/desorption isotherm of nitrogen adsorption using the BET equation. The results showed that a low dose irradiation of a solution with lower monomer concentration in methanol irradiated at high temperature and high dose rate resulted in a monolith with large pores. On the other hand, monolith with high surface area was obtained from a solution of higher monomer concentration in acetone irradiated with higher dose at low temperature and low dose rate. By the proper adjustment of these variables, monoliths with appropriate properties for a specific application can be prepared. Copolymerization of diethyleneglycole dimethacrylate with hydroxyethylacrilate resulted in monoliths of different hydrophilicity, while by copolymerization with glycidyl methacrylate epoxy functionalities were introduced.

THERMAL ANALYSIS OF CROWN ETHER COMPLEXES

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Development of crown ether structures capable of complexing organic ammonium cations is a significant research area as several biomolecules (like amino acids and some neurotransmitters) belong to this group of compounds. With the aid of a selective complexing agent it is possible to subtract these valuable substances from biological samples. Using a chiral crown ether ligand it becomes possible to separate the two enantiomers of chiral ammonium cations or to perform enantioselective reactions.

The examined complexes contain benzylammonium- [BA], (*R*)-(+)-α-phenylethylammonium- [(*R*)-PEA] and (*R*)-(+)-, and (*S*)-(-)-α-(1-naphthyl)ethylammonium perchlorate [(*R*)-NEA and (*S*)-NEA] salts as guests. In the cases of the BA and (*R*)-PEA an achiral pyridono-18-crown-6 ligand, and in the case of the (*R*)-NEA and (*S*)-NEA a chiral (*R*, *R*)-dimethylphenazino-18-crown-6 ligand [(*R*, *R*)-DMP18C6] were used as host molecules to obtain four different crown ether complexes.

Thermal analysis is a simple and fast method to obtain important information about the interactions between the complexes as well as between the host and the guest in the crystal structures. Desolvation processes, phase transitions (mainly melting and solid-solid phase transitions) and thermal decomposition processes were investigated.

Several thermoanalytical methods have been used including coupled techniques. TG/DTA-MS (Thermogravimetry/Differential Thermal Analysis – Mass Spectrometry) instrument provided informations about the mass loss during the heating of the sample (e.g. loss of solvent), and made the qualitative analysis of the decomposition products possible. Identification of the simultaneous and consecutive endo- and exothermic processes recorded with DSC (Differential Scanning

Calorimetry) technique was performed using HSM (Hot Stage Microscopy) measurements.

In all cases, the melting points of the complexes were much higher than those of both the host and the guest compounds, however, the decomposition of the complexes begins immediately after their melting is completed, while the salts and the crown ether ligands are thermally stable by 50 to 100 °C above their melting points. Strongly exothermic processes can be observed during the decompositions of the perchlorate salts and that of the complexes which are due to oxidative reactions of the perchlorate anion.

BA was observed to exhibit a reversible phase transition upon heating.

The heterochiral complex consisting of (*S*)-NEA and (*R*, *R*)-DMP18C6 shows an interesting thermal behaviour. It has a solid-solid phase transition followed by two melting points. HSM observations identified two different, simultaneously existing crystal modifications.

Acknowledgements

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MUTATION ANALYSIS OF THE ENTIRE KERATIN 5 AND KERATIN 14 GENES IN PATIENTS WITH EPIDERMOLYSIS BULLOSA SIMPLEX

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Introduction

The major structural proteins of the vertebrate epidermis and its appendages are keratins, members of the intermediate filament system. There are about 30 different keratin genes subdivided into two distinct sequence types (type I. and II. intermediate filament proteins), which are expressed in pairs in a tissue- and differentiation-specific fashion with complex expression patterns. The filament-forming capacity of a pair is dependent upon its intrinsic ability to self-assemble into coiled-coil heterodimers and their further polymerisation into the cytoskeleton system of the cells. Mutations in these heterodimer proteins perturb keratin filament assembly *in vitro*, and cause blistering human skin disorders *in vivo*. *Epidermolysis bullosa simplex* (EBS) is a group of blistering skin disorders caused by defects in one of the two keratin genes, *KRT5* and *KRT14*. The disease is typically inherited by an autosomal dominant way, although recessiveness has been also reported. Clinical manifestations are usually present at birth. All cases of EBS are characterized by mechanical stress-induced blistering of the skin, as a result of keratin filament clumping in the basal keratinocytes.

Aim of the Study

Our purpose was the disclosure of mutations underlying the clinical symptoms and comparison with skin histology in order to define phenotype-genotype correlation more precisely. Mutation analysis of *KRT5/14* genes in cases with the more severe Dowling Meara type-EBS (DM-EBS) and with the milder Weber Cockayne type-EBS (WC-EBS) was performed to determine the molecular genetic divergence in altogether 11 EBS patients.

Methods

DNA was isolated from peripheral blood lymphocytes of the probands and family members by SIGMA Miniprep Kit method. Next, the sequences of the functional *KRT5* and *KRT14* exons were amplified by PCR programs. For conformation-sensitive gel electrophoresis (CSGE) the PCR products were strained. The DNA samples having band shifts, were directly sequenced by an automated sequencer, and verified by restriction enzymes. For family screening, PCR products were also subjected to restriction endonuclease digestion.

Results

In this study, we have identified three novel dominant missence mutations (N123K, R125G and V133L) situated very close to each other in exon 1 of the *KRT14* gene. Both mutations are in the highly conserved residues of the *KRT14* gene. The V133L substitution in patient with WC-EBS phenotype is just outside the helix initiation motif. These data with new mutations render further information on genotype-phenotype correlation in EBS.

EXTRACTION OF SHIITAKE MUSHROOM WITH DIFFERENT SOLVENTS

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Considerable attention has recently been paid to healthy way of life and mainly to the importance of healthy nutrition intake. First of all it means fibre-rich and cholesterol-poor nutrition intake, on the other hand products free of solvent residues are more favourable. The Supercritical Fluid Extraction (SFE) is one of the separation processes which produces products free of solvent residues as heat-sensitive compounds can be extracted and is an environmentally accepted technology.

The goal of my work was to extract valuable components and natural agents from the shiitake mushroom (*Lentinus edodes*). Laboratory and pilot plant extractions were carried out using different solvents.

For the laboratory Soxhlet extraction of shiitake four different solvents (*n*-hexane, ethyl-acetate, isopropyl-alcohol, ethyl-alcohol) were applied, all of them are registered as 'safe' regarding to EU policy. Increasing the polarity of solvents higher extraction yields were achieved (*n*-hexane: 1.03%; ethyl-acetate: 4.42%; isopropyl-alcohol: 15.49%; ethyl-alcohol: 30.79%).

Pilot plant experiments were also performed in Soxhlet extractor with ethyl-alcohol (96% of purity) and by CO_2 in 5 l high-pressure vessel at 50 °C and 450 bar.

These parameters were the case obtaining the highest extraction yield from shiitake containing all the soluble compounds. The extraction yield was low (0.530%) similar to the yield obtained by *n*-hexane according to the same solubility properties. With SFE only the one sixtieth part of the yield of ethanol extraction was achieved.

The high valued immune-stimulating compound, lentinane can be extracted by water, while the strong, aromatic compounds (sulfure containing) can be eliminated by SFE.

Our further aim is to optimize the extraction of shiitake to obtain a product which contains lentinane in active form and where the presence of aromatic compounds is moderated.

DETERMINATION OF MICRO-POLLUTANTS IN THE THE DANUBE OF THE RÁCKEVE-SOROKSÁRI BRANCH

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Besides regularly controlled pollutants there is an increasing number of organic compounds having extremely harmful effects on natural waters even in very low concentration. Surface waters are source of drinking water. Any pollution in surface waters will influence the quality of tap water too. It is impossible to control all harmful organics in the surface water, but some selected pollutants and their degradation products must be measured regularly. In the international literature these are known as micro-organic pollutants. Their determination is recommended by the Environmental Agency of the European Union, but until now, there is no regulation on determination of organic micro pollutants in Hungarian environmental law, which was the reason to start controlling these compounds in Ráckeve-Soroksári Danube branch (Ráckevei-Soroksári – Dunaág). The measured data are used to create a database. Wide range of pollutants can be found under the name of micro pollutants, for example volatile compounds (VOC), residue of crude oil, detergents and their degradation products, pesticides, polycyclic aromatic hydrocarbons (PAH) and phenols.

List of Pollutants Measured:

Volatile compounds: benzene, toluene, (o-, m-, p-) xylenes, 1,2,4-trichlorbenzene, 1,3,5-trichlorbenzene, 1,2,3-trichlorbenzene,

Hexachlorbenzene-pesticides: Alakchlor, Chlorpiriphos, Chlorfenvinphos, Simazon, Atrazin, Diuron, Izoproturon, Lindan, Endosulfane

Polycyclic aromatic hydrocarbons (*PAH*): naphtene, acenaphtylene, benz(a)anthracene, chrysene, benz(k)fluoranthene, benzo(a)pyrene, dibenzo(a,k)anthracene, benz(g,h,i)perylene indeno(1,2,3- c,d)pyrene

Detergent derivatives: 4-octil phenol, 4-nonil phenol.

To analyse these compounds we took water samples on 10 spots in the Danube upstream from the Gubacsi Bridge along the bank. Enrichment of the samples was carried out with SPE method (Sep Pack Plus C-18 column). The concentrated samples have been examined with various methods.

The Pollutants and Methods Applied are Given Bellow:

Pollutant	Method applied
PAH	HPLC-FL
Pesticides	HPLC-UV, GC-MS
Alkylphenols	HPLC-FL
VOC	HS-GC

For pesticides, the quality of the mentioned reach of the Danube is acceptable since their concentrations do not even reach the threshold of the permissible load of underground water. We met salient values only in case of a few compounds which are: isoproturon, a selective herbicide, used for perennial plants and corns. The source of these compounds is the modern agriculture or the redissolving from soil polluted with pesticides. The areas this flow is going across and the activity (agricultural, industrial) that is done around the catchment areas should be mapped. Chlorpiriphos has widespread usage against grub, insects, flies and other pests. It also proves, similarly to the former compounds, strongly chemicalised farming. For heavy PAH load, the examined reach of the Danube is acceptable. It is especially important, that the proportion of benz(a)pyrine has always been under the limit value (10 mg/m^3). As to light PAH, the concentration of fluorene, fenantrene, fluorentene has been between the pollution limit value and the meddling limit value. These compounds refer to earlier oil pollution. Another evidence for that is the concentration of the easily biodegradable naftaline being under meddling value. As these regulations are given only for underground water, these values can be considered as tolerable. It is concluded, on the basis of the measured data, that the PAH load is acceptable in the investigated area. For 4-octyl phenol and 4-nonil phenol there are no limit values in the literature. They come from the alkyl-phenol detergents used earlier. Their amounts are small, negligible; they are likely to have got into the river with earlier pollutants and are continuously redissolving. As for their concentration the water quality of the Danube is acceptable. The concentration of VOC compounds were between 1–10 ppm which shows that quantity of aromatic and chlorinated hydrocarbons is small. Concerning the investigated pollutants in the mentioned reach of the river Danube the quality of water is tolerable.

EXAMINATION OF THE 1H → 2H SIGMATROPIC REARRANGEMENT OF 2,4,6-TRIALKYLPHENYLPHOSPHOLES [1]

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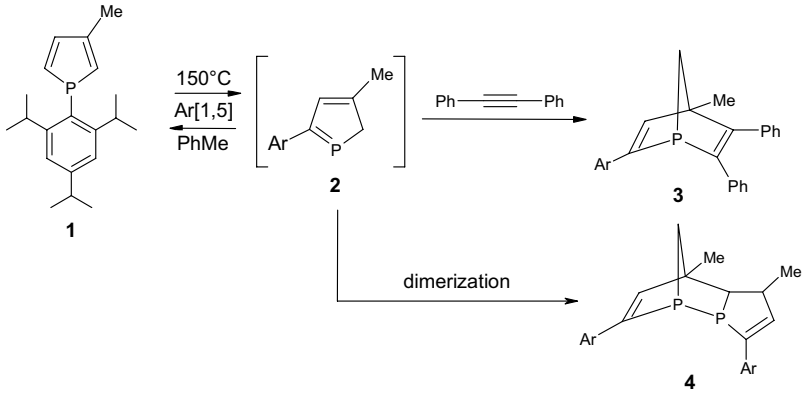
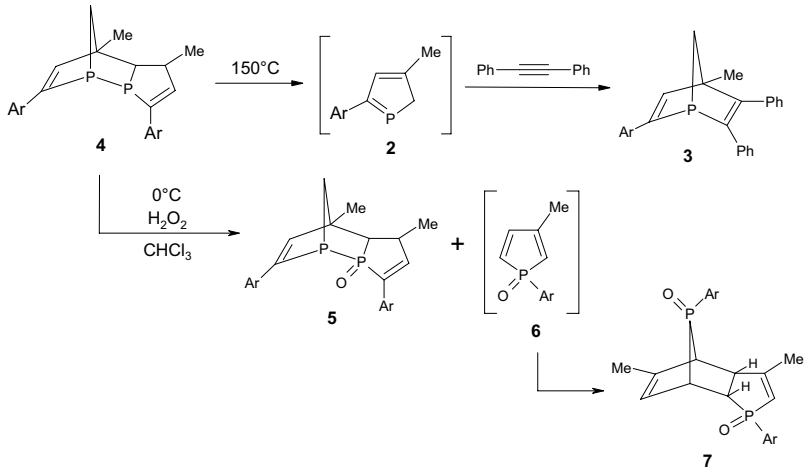
2,4,6-Trialkylphenylphospholes introduced recently, form a special class of phospholes, as they have some aromatic character due to the planarization of the P-pyramid. Despite their moderate aromatic character, we found that they underwent a sigmatropic Ar[1, 5] rearrangement on heating at 150 ˚C in toluene, in a sealed tube. The intermediate 2H-phospholes (**2**) were trapped by reaction with tolane to furnish 1-phosphanorbornadienes (**3**). Thermal treatment of arylphosphole (**1**) in the absence of tolane led to the formation of a cycloadduct that was dimer (**4**).



1 **2** **3** **4**

Reversibility for the formation of dimer (**4**) was proved first by its reaction with tolane, as it led to the formation of cycloadduct (**3**). At 150°C, the dimer was decomposed to two units of 2H-phosphole (**2**) entering into reaction with tolane. It is the novel and quite surprising observation of ours that precursor (**3**) was also dedimerised on treatment at 0 ˚C with hydrogen peroxide, as it afforded the hemioxide derivate (**5**) and the phosphole oxide intermediate (**6**) leading spontaneously to its dimer (**7**).



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QUICK ANALYSIS METHODS IN THE QUALITY CONTROL OF CORN PROCESSING

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Corn processing requires up-to-date enzyme technology, minimalization of waste and also requires safe working conditions and favourably, quick controlling systems should be devised and introduced.

On the one hand, quick analysis methods are able to partly replace classical methods and on the other, they are able to meet all the requirements of the latest analytical ones.

Hungrana Corn Processing Plant (based in Szabadegyháza) provided the means for me, thus I examined the application of quality controlling methods, which are quick and can be easely fit in industrial environment.

According to the needs of the plant, I analysed the quality changes of raw material on the long run and revealed the possible reasons for the adverse quality changes caused by the growing circumstances (starch content loss or fluctuation, and growth of protein content).

Comparing NIR spectoscopical and traditional chemical methods I statistically analysed and altered calibration models that are to classify dry raw maize.

I worked out near infrared spectroscopic calibrations to grade wet raw maize. Analysing these methods I proved to what extend does the accuracy of the reference methods influence the capability of the calibrations.

To be able to reveal the microbiological infection of raw maize quickly, I examined the usability of the so-called ‘electornic nose’ – which is an analysing method.

Applying the immun chemical method (ELISA, Veratox DON 5/5) I revealed that the processing plant’s being infected, mainly in respect of byproducts, which cannot be ignored.

Immune based test-strip method (Agri-Screen Cry 9C Strip test) proved that maize samples examined, were not genetically modified ones (in excess of 0.125%).

HETEROGENEOUS CATALYTIC ASYMMETRIC HYDROGENATIONS

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Asymmetric syntheses can be carried out with heterogeneous catalysts. In hydrogenations simple adding of chiral compounds to the reaction mixture can have enantioselective effect. If the addition is made in catalytic amount, these compounds are called chiral modifiers.

There are two catalyst/modifier/substrate systems which produce almost total optical purity: hydrogenation of β-keto esters on Raney-Ni catalysts modified with tartaric acid, and hydrogenation of α-keto esters on platinum catalysts modified with cinchona alkaloids. Similarly to enzymatic reactions, such effects are highly specific to the reactant, chiral modifier and catalyst.

Research has been carried out at the Department of Chemical Technology for long time, studying enantioselective heterogeneous catalytic hydrogenations. The two model reactions are: the reduction of the carbonyl group in ethyl pyruvate on modified platinum catalysts, and hydrogenation of the C=C bond of isophorone on modified palladium catalysts. The aim of experiments is to achieve higher enantioselectivity, to explore the working mode of these systems, and as further result to discover new, effective synthetic methods.

Nine compounds have been tested containing chiral moieties as cinchonidine, trimethyl-1-aminomethyl-2oxa-bicycloheptan-2-on, and (S)-diphenyl-pirrolidine-methanol (DPPM) as new, potencial chiral modifiers. The effects of modifiers structure, solvent, and concentrations were investigated.

One of the above mentioned chiral lactons, that has 1-naphtyl moiety on the basic nitrogen atom, may act as chiral modifier. Using that compound resulted in 9% excess of S enantiomer for ethyl lactate and in 19.3% excess of R enantiomer for dihydroisophorone, though at the last one the conversion rate was rather low.

Two DPPM moieties coupled with 2,6-dimethylpyridin, being the best among the tested molecules, gave 18.6% excess of R enantiomer for ethyl lactate, 14.4% excess of S enantiomer for dihydroisophorone. For optimisation of the reaction conditions further experiments are required.

COMPARATIVE ANALYSIS OF GENE BANK CARP SPECIES WITH RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

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Retaining the biological diversity; gene preservation is one of the areas of environment protection with outstanding significance. In livestock-breeding in order to select the species which are adequate to the consumer and economic expectations and to the changing environmental and technological relations, it is needed to know and to maintain the existing diversity of species. In this project gene banks have a major role. During my work in the genetic department of the Institute of Feedstuff and Small Livestock Breeding, I could do the genetic comparison of the domestic and foreign species maintained by the world's largest carp gene collection, the gene bank of Szarvas. Apart from the usage of the traditional phenotype or biochemical polymorphisms, the knowledge in molecular biology and laboratory technologies of our days also enables origin controlling on DNA basis. In this dissertation I used RAPD-PCR (polymorphic DNA mainfolded by random polymer chain reaction) technology and isolated the DNA markers distinguishing the 19 species of carp of the gene bank, and examined the relation of affinity.

RESOLUTION OF RACEMIC 1-PHENYL-1-PROPANOL

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Nowadays the preparation of chiral compounds in optically active forms plays a dinamically increasing role in the pharmaceutical process and in the research, too. Among the methods for the preparation of optically active compounds the resolution by separation of diastereomeric salts has become widely used. In addition, in the last years biocatalysis has become more and more important, because it is an environmental-friendly and very selective method.

We examined the resolution of racemic 1-phenyl-1-propanol with both methods and we found that the lipase-catalysed kinetic resolution resulted better enantiomer-separation than the chemical method based on the separaton of diastereomeric salts. Our results are the following:

- The lipase-catalysed kinetic resolution of the racemic alcohol has been tested with various, commercially available enzymes and enantiomer-separation has been achieved with PfL, Amano AK, Amano PS, Amano PS-C, PpL, CcL, and Novozym 435 enzymes. In these experiments vinyl acetate was the acylating agent and the solvent, too (*Fig. 1*):

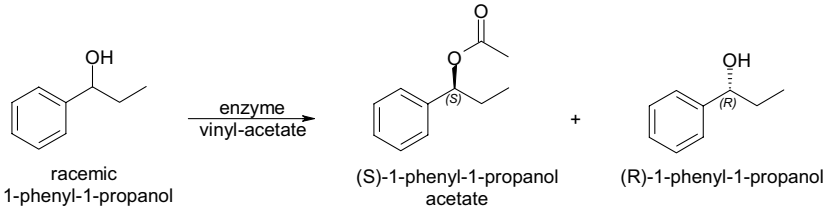

Fig. 1.

  The best result was obtained with Amano AK, Amano PS-C and Novozym 435 enzymes.
- The effect of the solvent and acylating agent has been examined with the two choosen enzymes (Amano AK and Novozym 435) for the enantiomer-separation.
- The maleinic, succinic and the phthalic mono esters of the racemic alcohol have been synthesized and the resolution has been tested using different chiral

bases (*Fig. 2*). Enantiomer separation was obtained with (S)-(-)-1-phenyl-ethylamine and cinchonide in case of the maleinic and the succinic mono esters.

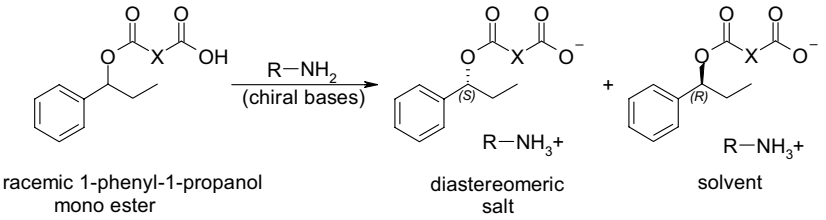

Fig. 2.

SPECTROSCOPIC TECHNIQUES FOR STRUCTURAL ANALYSIS OF THE RETROVIAL NUCLEIOAPSID-dUTPase FUSION PROTEIN

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The ubiquitous enzyme dUTP pyrophosphatase (dUTPase) is unique in its capacity to prevent incorporation of uracil into DNA. dUTPase produces the dTTP precursor dUMP and decreases cellular dUTP levels, its lack leads to an elevated dUTP/dTTP ratio and DNA with a high content of uracil. Excision repair of uracil-DNA results in futile cycle because of the low cellular dTTP content. Subsequently, multiple double-stranded DNA break and thymine-less cell death occur. dUTPase is essential in both pro- and eukaryotes and restricts host range and pathogenicity in both retroviruses and *Herpesvirus.* Null mutations in retroviral dUTPase gene affect viral growth only in non-dividing cells. Consequently, viral dUTPases are subjects of medical interest.

Retroviral dUTPase genes are located in non-primate lentiviruses and betaretroviruses at different genomic locations. Lentiviral dUTPase genes are in the *pol* open reading frame between reverse transcriptase and integrase genes, although in betaretroviruses, the 5'-portion of the frame encodes dUTPase. Betaretroviruses encode dUTPase in the *pro* open reading frame located between *gag* and *pol*. Ribosomal frameshifts during expression of retroviral proteins provide a unique possibility for covalent joining of nucleocapsid (NC) and dUTPase within Gag-Pro polyproeins. The NC-dUTPase fusion protein exists both within the virions and infected cells providing the only form of dUTPase.

Oligonucleotide and Zn^{2+} binding is well retained in the fusion protein, which is the first example of acquisition of a functional nucleic acid binding module by the DNA repair factor dUTPase. Binding of the octanucleotide (TG_4) to NC-dUTPase modulates enzymatic function, indicating that the low catalytic activity may be compensated by adequate localizaton.

In the present work, sensitive spectroscopic techniques were evaluated for investigation of the binding interactions between both the dUTPase and the NC regions and their cognate ligands (oligonucleotide (TG_4) and Zn^{2+}, as well as dUTP analogue, respectively). I studied the MPMV NC-dUTPase fusion protein and its complexes using fluorescence and circular dichroism spectroscopies.
The results:

- Fluorescence spectroscopy proves to be a reliable, specific method to examine the complex formation between the Zn^{2+} ion and the NC segment in NC-dUTPase.
- Analysis of fluorescence emission spectra of the fusion NC-dUTPase as compared to the dUTPase. Lacking the NC segment identifies a characteristic

difference in the microenvironment of the tryptophan side chains in these two proteins. Based on this observation, this method will be used for detailed investigation of the nature of microenvironmental changes around the tryptophane side chains as induced by interaction with the cognate ligands.

- A comparison between fluorescence and circular dichroism spectroscopies with respect to the investigation of oligonucleotide – protein interactions revealed that the latter technique is better suited for this approach. Circular dichroism has also the advantage of providing some low-resolution structural information that might be classified later as modulation of beta structure, alpha helices or turn conformations.

DEVELOPMENT OF A METHOD FOR ESTIMATING THE AMOUNTS OF THE DIFFERENTLY BIODEGRADABLE ORGANIC FRACTIONS IN WASTEWATER

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It is highly important to broaden and improve wastewater treatment in Hungary not only in order to protect and restore the quality of the natural water-bases, but also as a requirement of the European Union. This can be accomplished cost-efficiently by using the adequate technology to the given wastewater quality and effluent requirements. The conventional characterisation uses parameters like COD (Chemical Oxygen Demand) and BOD_5 (Biochemical Oxygen Demand) for measuring the organic content of wastewater. These parameters do not provide information on the differently biodegradable fractions (readily, slowly, not at all).

The studies are part of the research project 'Development of Advanced Wastewater Treatment Systems to Meet EU Requirements' funded by the Hungarian National Research and Development Program, and are focused on the development of new, comprehensive methods being well applicable in local circumstances both in design and operation of wastewater treatment plants. During the research different approaches: denitrification tests, and BOD measurements were applied.

The denitrification test as a method to achieve the goals has numerous advantages. Firstly, it can be carried out with much lower cost than the generally used respiromertic methods, and it is also less complicated, and therefore needs less expertise. Secondly, it measures the amount of denitrifiable organic matter, which is critical in plant design, because of the strict effluent N criteria. Denitrifiable organic matter is crucial, because only this fraction can be degraded under anoxic conditions, so its amount determines the capacity of nitrogen removal in pre-denitrifying systems. It proved to be favourable to apply the two methods together, and to develop a new way for the evaluation of data acquired from BOD test systems, which is similar to that used in respirometric measurements.

By comparing the results driving from the two methods it could be concluded that they estimate the amount of readily biodegradable substrate similarly. This indicates that both the denitrification tests and BOD measurements with the new data evaluation method can be applied safely to estimate the amount of different organic fractions of wastewater.

The way of further development of the methods discussed leads through the simultaneous application of two denitrifying systems with different sludge concentrations. This would lead to a more precise assessment of the readily biodegradable fraction, and to a significantly shorter experimental time required for estimating the total amount of biodegradable substances.

THE SYNTHESIS OF NOVEL FLUORESCENT MONOAZA CROWN ETHERS HAVING ACRIDONE UNIT BOUND TO THE NITROGEN ATOM OF THE MACRORING BY A METHYLENE SPACER

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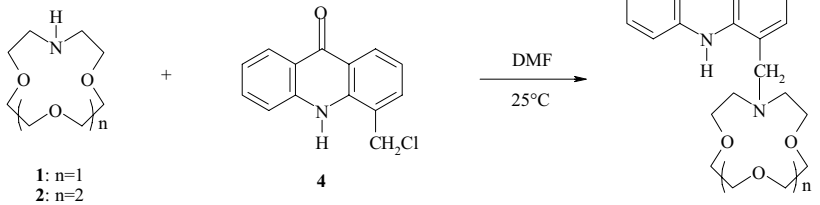
Macrocycles that possess ionophore ability have been the subject of wide investigations not only for their synthesis but also for their numerous technological and analytical applications since their discovery by C. J. Pedersen. These type of macrocycles can be used for studying the natural phenomenon of molecular recognition on relatively simple synthetic models. Cationic recognition studies on chromophore macrocycles by optical spectroscopy have increased because of interest in inorganic cationic chemistry. Determination of different metal cations can be performed with these macrocycles because of their selective ion-binding ability. The great advantage of crown ethers containing flurophore moiety is that they can be studied by fluorescence spectroscopy. Among the variety of optical methods used in analytical spectroscopy, fluorescence plays a major role because of its intrinsic sensivity, good selectivity and versatility.

We synthesized three novel fluorescent crown ethers having a fluorophore group attached by a methylene spacer in order to examine their cation-binding properties by fluorescence spectroscopy.

We bound an acridone derivative to monoaza-12-crown-4 (**1**), monoaza-15-crown-5 (**2**) and monoaza-18-crown-6 (**3**) ethers. Our work has involved the preparation of the two direct precursors, monoaza crown ethers (**1**, **2** and **3**) and 4-chloromethylacridine-9(10*H*)-one (**4**), too. Monoaza crown ethers **1**, **2** and **3** were synthesized by a new method. We used the acridone unit to build in a fluorophore group. The appropriate acridone derivative (**4**) was prepared by a five-step procedure. We bound the latter compound (**4**) to the monoaza crown ethers by *N*-alkylation to obtain macrocycles **5**, **6** and **7**.



1: n=1
2: n=2
3: n=3

4

5: n=1
6: n=2
7: n=3

RESOLVING OF CHIRAL ALCOHOLS BY SUPERCRITICAL EXTRACTION

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The biological effect of enantiomers of chiral molecules can be significantly different. Resolution is one of the possible ways for separating enantiomers. Many times separation of enantiomers by diastereomeric compounds needs organic solvents.

Resolution of racemic *trans*-2-halogen-cyclohexane-1-ols (Cl, Br, J) and 2-isopropyl-5-methylcyclohexane-1-ol (menthol) with supercritical CO_2 extraction was studied in this work.
(-)-(2R,3R) $-$ O, O'-dibenzoyl-tartaric acid monohydrate was used as resolving agent.

Two methods of resolution were used. First *trans*-2-halogen-cyclohexane-1-ols were examined using organic solvent at the sample preparation and decomposition of diastereomeric comlpex. It was found that the highest enantiomeric excess (ee), as the optimal resolving agent/racemic alcohol molar ratio (mr) increased with the size of halogen substituent (extract: $ee_{Cl} = -72.2\%$; $ee_{Br} = -89.3\%$; $ee_J > -99\%$; raffinate: $ee_{Cl} = 64.4\%$; $ee_{Br} = 91.9\%$; $ee_J = 97.9\%$; optimal molar ratio: $mr_{Cl} = 0.55$; $mr_{Br} = 0.63$; $mr_J = 0.67$). The effect of extraction pressure and temperature was examined. The effect of pressure increased with the size of halogen substituent.

Fractionated extraction was used as another resolving method. It does not need any organic solvent during the resolution process and the diastereomeric complex could be decomposed in the extractor. Thus both enantiomers are obtainable in the separator. The value of mr was the same as in the previous resolving method, but temperature had significant effect also. The best enantiomer separation was obtained at 100 bar, 33 °C; (extract: $ee_{Cl} = -48.4\%$; raffinate: $ee_{Cl} = 65.1\%$).

Resolving enantiomers of menthol was examined by fractionated extraction. The diastereomeric complex of menthol was continously decomposed during the extraction process (highest ee $= -73.8\%$). Further decreasing of extraction pressure and temperature could lead to stop the decomposition.

SYNTHESIS, CHARACTERIZATION AND APPLICATION AS TAGGING MOLECULES OF NOVEL CHROMO- AND FLUOROGENIC LIPOPHILIC ANIONS

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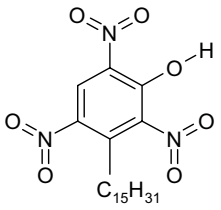
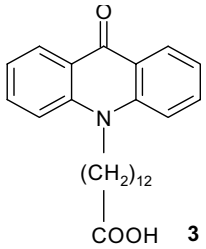
The use of various tagging molecules (markers) has found a widespread application in the field of analytical chemistry. Markers are compounds which show the presence of other molecules directly non-detectable by the given analytical method by establishing an unambiguous relationship with the occurrence or concentration of the latters. Huszthy and co-workers have long been dealing with the synthesis of acridone derivatives having very advantageous chromo- and fluorogenic properties.



1



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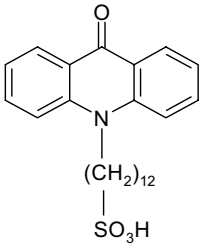

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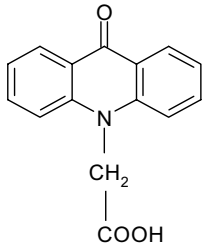

4

During my research work I have synthesized the compounds shown above with definite analytical applications. These are as follows: 3-pentadecyl-2,4,6-

trinitrophenol (**1**), 12-(9-oxo-10*H*-acridine-10-yl)-dodecanesulphonic acid (**2**), 12-(9-oxo-10*H*-acridine-10-yl)-dodecanoic acid (**3**) and (9-oxo-10*H*-acridine-10-yl)-acetic acid (**4**). Chromo- and fluorogenic anions **1** and **2** with lipophilic character have been used as markers for examining the cationic flux across the membranes of ion-selective electrodes and the acridone derivatives containing a carboxylic group (**3** and **4**) are being used in fluoroscence tagging of peptides.

PROTONATION STUDY OF 2,4,6-TIAMINOPYRIMIDINES AND DERIVATIVES

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The aim of this work was to study the steric and electronic effects of protonation of aminopyrimidines using quantum-chemical computation.

Quantum-chemical calculations were carried out with the B3LYP/cc-pVDZ level. The geometry, Mulliken-charges, electron energies and NICS-indexes were computed.

- It was found that in the C(5) protonated form the bonds are of tetrahedral orientation, and longer than in the base form. The structure of C(5) protonated form shows that amine/dimethylamine groups are in the plane of the ring.
- The results of the Mulliken-charges show that in the ring the C(5) atom is also a negative centrum, and in the C(5) protonated form the positive charge is shared equally by the atoms in the molecule – the charge of amine/dimethylamine groups are increased.
- It was confirmed with NICS-indices that both the unprotonated basis and nitrogen protonated forms are equally aromatic but the C(5) protonated forms are not aromatic.
- In the HOMO of basis the largest coefficient is on the C(5) atom which is in line with the observed reactivity.
- The computed proton affinity was in good qualitative agreement with the rate of protonation observed from NMR data.

Our further plan is to repeat the calculations taking into consideration the solution phase.

THE INTERACTING PROTEIN NETWORK OF duTPase: ANALYSIS BY IMMUNOLOGICAL METHODS

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Abstract

Faithful conservation, replication, and transmission of the genetic information is essential for all living organisms. The enzyme dUTPase prevents uracil incorporation into DNA by hydrolysation of dUTP into dUMP and anorganic pyrophosphate, and therefore contributes a unique and essential preventive DNA repair function to the variety of DNA repair mechanisms. Lack of dUTPase activity leads to uracil-substituted DNA. High uracil-content of DNA induces DNA fragmentation and thymine-less apoptosis of the cell. dUTPase antagonism can therefore provide a promising novel antiviral and anticancer therapeutic strategy.

The present work is connected to investigation of the interacting macromolecular partners of the dUTPase in *Drosophila melanogaster*, an organism widely used and genetically well-characterized for developmental studies. To study the interacting protein network of dUTPase, I designed and adopted two different methods capable of identification and follow-up of cellular protein partners of the enzyme. Another aim was to investigate the level of the dUTPase enzyme expression in Drosophila S2 cells.

Results

1. In far-Western analysis, the proteins of the Drosophila S2 cell line extract were applied to SDS-PAGE and transferred to nitrocellulose membrane. Proteins were visualized by immuno-staining followed by enhanced chemiluminescence.
2. Using co-immunoprecipitation, dUTPase and its interacting partners were obtained from S2 cell extract and separated on SDS-PAGE. Gel slices were cut out for mass-spectrometry analysis and identification.

I successfully purified polyclonal antibody for Drosophila dUTPase by immunoaffinity- chromatography and used it in further investigations. I demonstrated the presence of dUTPase interacting partners in Drosophila embryos and larvae by far-Western analysis. One of the partners appears at 60 kDa on SDS-PAGE,

corresponding to the apparent molecular mass of a previously proposed dUTPase inhibitor protein. The expression of this protein is connected to the first larval stage, as shown in my experiments and also in the previous report. I also detected some less intensive, but specific bands in the embryonic extract. Patterns of the interacting partners of dUTPase are shown to be different in the diverse developmental stages (embryo and larva).

We documented that the expression of dUTPase in Drosophila depends on cell cycle in case of both isoenzymes, in contrast to the human case where this holds only for the nuclear isoform.

ESTABLISHING TRENDY COLOURS BY MEANS OF TRICHROMATIC DYE MIXTURES

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Jantai Tamásné
Anilin Trading Company

Trendy (fashion) colours have been available from French and/or British fashion centres.

Four trichromatic dye combinations were selected in the laboratory of Anilin Trading Company for our experiments.

Five dye systems were selected for trichromatic dyeings in the laboratory of the Anilin Trading Company. Three of them were made of reactive dyes for cotton, one from disperse dyes for PES and another one from cationic dyes for PAN.

Four dyeing procedures were foreseen for the production of dyed samples. 790 computer calculated recipes were made by means of colour matching for 158 trendy colours with 5 trichromatic systems. The accuracy of reproducibility (colour difference and metamerism) for all the 158 trendy colours was also the result of the mentioned colour matching. The highest accepted value of metamerism was 2. Sets of reproducible trendy colours were selected allowing colour difference of 0, 0.5, 1 and 2, respectively.

If the accepted colour difference was zero ($\Delta E^*_{ab} = 0$) than the percentages of reproducible trendy colours were 27 for PES, 23 for PAN and 39 for cotton. If, however, $\Delta E^*_{ab} = 0.5$ was allowed the percentages of the reproducible trendy colours were nearly equal to each other, namely 52 for PES, 57 for PAN and 58 for cotton. The achievable reproducibility for trendy colours of men's wear is usually better than that of women's wear.

E.g. the accuracy for PAN men's wear of trendy colours studied in the autumn – winter season in 2000–2001 was 100%. The respective accuracy for women's wear was 83 %.

No significant increase in accuracy of trendy colour reproduction could be achieved if the colour difference was allowed up to $\Delta E^*_{ab} = 2$.

Subsequently to colour matching measurements laboratory dyeing experiments were performed. Cotton and PES fabric samples were dyed for selected trendy colours.

From the 2002–2003 season 8 trendy colours were selected from 18 colours prescribed for autumn–winter; another 8 from 18 colours prescribed for spring–summer. The allowed accuracy for reproduction was $\Delta E^*_{ab} \leq 1$ and for Metamerism ≤ 1.

All 16 selected trendy colours could be reproduced within the prescribed accuracy. In the case of cotton dyeings the procedure had to be modified 5 times whereas in PES dyeings only 3 times.

MECHANISTIC INVESTIGATION OF PHENYLALANINE AMMONIA-LYASE WITH *N*-METHYLATED PHENYALANINES

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Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) is an important plant enzyme that catalyses the non-oxidative elimination of ammonia from L-phenylalanine in the first step of its metabolism. Because of its central role in plant metabolism PAL is a potential target for herbicides.

The aim of our study was to investigate *N*-methyl-L-phenylalanine, *N*-methyl-4-nitro-L-phenylalanine and *N*, *N*-dimethyl-4-nitro-L-phenylalanine as substrates or inhibitors of phenylalanine ammonia-lyase from *Petroselinum crispum* to provide further experimental details to the mechanism of action of this enzyme. Whereas the first compound was a reluctant substrate ($K_m = 6.6$ mM, $k_{\text{cat}} = 0.22$ s-1), no reverse reaction could be detected using methylamine and (*E*)-cinnamate. The K_m value for ammonia in the reverse reaction using (*E*)-cinnamate was determined to be 4.4 and 2.6 M at pH values 8.8 and 10, respectively. This implies, that in the reverse reaction the concentration of the ammonia and not the ammonium ion is decisive. The *N*-methyl- and *N*, *N*-dimethyl-4-nitro-L-phenylalanines showed only strong inhibitory effects ($K_i = 130$ nM and 8 nM, respectively). These results were also rationalized by molecular mechanics calculations within the active site model of the PAL and were discussed in terms of the mechanism of action of phenyalalanine ammonia-lyase.

MODELLING THE TRANSITION BETWEEN MITOTIC AND MEIOTIC DIFFERENTIATION IN FISSION YEAST

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The fundamental goal of post-genomic cell biology in the XXI. century is to clear out how the molecules, that build up cells, control a living, reproducing cell. Mathematical modelling provides efficient tools to understand the function of complicated protein networks working in cells. I worked on the transition between asexual and sexual development in fission yeast. I based my model on information gathered from physiological experiments of the '70s to the latest DNA-microarray analysis. By using these data I constructed a mathematical model of the network responsible for the regulation of meiosis initiation and examined it by computational simulations and phase plain techniques. After the analysis of the dynamical features of the model I pointed out the fact that the regulation of transition between mitosis and meiosis rests on the antagonistic interaction of two enzymes. In addition with my model I could explain the behaviour of some mutants of the regulatory network.

APPLYING A NEW SYNTHESIS STRATEGY TO PRODUCE 15-β-HYDROXYVINCADIFFORMINE

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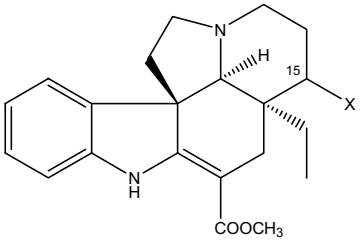
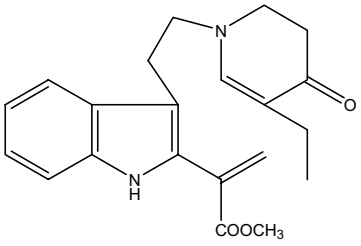
A new convergent synthesis strategy has been developed for building up the aspidospermane skeleton at the Budapest University of Technology and Economics, Department for Organic Chemistry, Research Group for Alkaloid Chemistry. In the procedure, an appropriately formed tryptamine derivative, as common starting material, was allowed to react with adequately built-up aldehydes or aldehyde-equvivalents; as a result, tetracyclic esters were obtained from which the target compound could be produced in several steps. Based on this strategy, the objective was set to produce 15-β-hydroxyvincadifformine (1).



1 X: -OH
2 X: =O



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The tryptamine derivative containing a secondary amino group, as key molecule of the convergent synthesis, was allowed to react with an appropriately formed aldehyde-equivalent. However, the reaction did not result in the product expected, but in an activated enamine with a terminal carbon-carbon double bond. In knowledge of the result, it seemed to be reasonable to let the aldehyde-equivalent react with the tryptamine derivative containing a primary amino group.

The conception was successful, the mixture of 15-oxovincadifformine (2) and 15-oxo-$\Delta^{20(21)}$-secodine (3) was obtained. Reaction of dihydrooxosecodine (3) in xylol in presence of *p*-toluenesulphonic acid monohydrate resulted in 15-oxovincadifformine (2). By stereoselective reduction of the carbonyl group, the 15-β-hydroxyvincadifformine (1) was reached.

PHOTOPHYSICAL PROPERTIES OF OXAZINE 1 – *p*-SULFONATOCALIX[8]ARENE SUPRAMOLECULAR SYSTEMS

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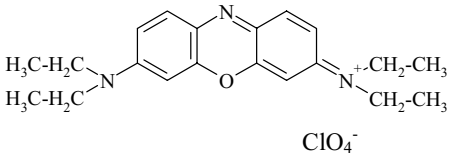
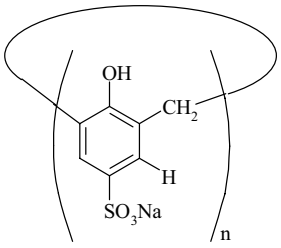
Fluorescent dyes are widely applied in biological and in material science as sensitive probes, providing information about their microenvironments. The behaviour of dye probes in various environments can be studied on model systems, like host-guest complexes, micelles, vesicules or aggregates with proteins. In our work the photophysical properties of the fluorescent dye oxazine 1 (**OX**, perchlorate salt) has been studied in the presence of the supramolecular host *p*-sulfonated calix[8]arene (**SCX**, sodium salt) in aqueous solution. Steady-state absorption and fluorescence spectroscopy and fluorescence lifetime measurements have been applied as experimental methods.

The absorption spectra gave clear evidence that **OX** and **SCX** form two supramolecular complexes, **OX** · **SCX** and $\mathbf{OX}_2$ · **SCX**, in consecutive reactions. The quantitative analysis of the spectra yielded the values for the respective equilibrium constants and the spectra of the two complexes, which are not available in pure state in solution. The steady-state fluorescence spectra and the fluorescence decay curves could not be interpreted applying this simple reaction scheme. The fluorescence properties of systems containing **SCX** in high excesses suggest that the emission is sensitive to the concentration of the Na^+ ions and to the degree of deprotonation of the phenolic OH-groups.



OX



n = 8

SCX

Competition processes between fluorescent dyes and non-fluorescent substrates for binding sites are of great significance in analytical chemistry. The behaviour of **OX** in such competitive reactions has been studied by measuring the absorption and fluorescence spectra of a selected **OX** – **SCX** system, following the addition of $(CH_3)_4N \cdot HSO_4$, as a salt of a competing quaternary ammonium ion, in various concentrations.

STRUCTURE ANALYSIS OF OLIGOPEPTIDES BY MEANS OF QUANTUM CHEMICAL CALCULATIONS

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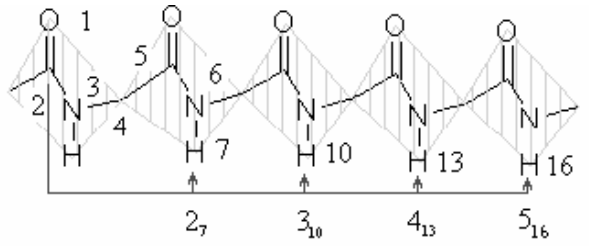
The secondary structure of proteins is of primary importance in the biological functions of these molecules. Our present knowledge on the characteristics of the secondary structures stems from X-ray and NMR studies as well as from low-level molecular modelling. Each listed method has its deficiencies, due to that our information on the exact character of the interactions stabilising the secondary structures is still incomplete. Sophisticated quantum chemical calculations are well suited to study weak molecular interactions, hence to explore the nature of the secondary structures of oligopeptides. The goal of our present study was to elucidate the role of hydrogen bonds in various oligopeptide conformers, viz. β-sheet, 2_7-ribbon, 3_{10}-helix, α-helix (on the figure 4_{13}), π-helix (on the figure 5_{16}), β-turn II and γ-turn. For the present calculations hexapeptides of glicine and alanine were selected. The effect of solvent (water) was investigated using the IPCM model.



Secondary structures listed above were optimised at the B3-LYP/6-31G** level. The strongest intramolecular hydrogen bonds have been obtained in the 2_7-ribbon structure among the investigated conformers. However, we did not find a straight correlation between the number/strength of the hydrogen bonds and the relative stability of the secondary structures. This indicates, that the dipole-dipole interactions and steric effects play a role in magnitude similar to that of hydrogen bonding in stabilising the conformers. The energy ordering obtained in vacuo is disturbed upon the effect of water: most noteworthy is the strong stabilization of α-helix with respect to the other conformers.

We determined the geometrical characteristics of the various secondary structures: this included the range of ϕ and ψ torsion angles, the charateristic lengthening

of the C=O and N–H bonds involved in hydrogen bonding and the shortening of the C–N bonds. An excellent correlation was found between the lengthening of the N–H bonds and the lengths of the hydrogen bonds. The correlation is much poorer with the lengthening of the C=O bonds, which has a strong effect also from hyperconjugation interactions. The methyl substituent in alanine effects mainly the character of the β-sheet which is nearly planar in oligoglycines.

REGIOSELECTIVE METALATION OF 1-(METHYLPHENYL)PYRROLES

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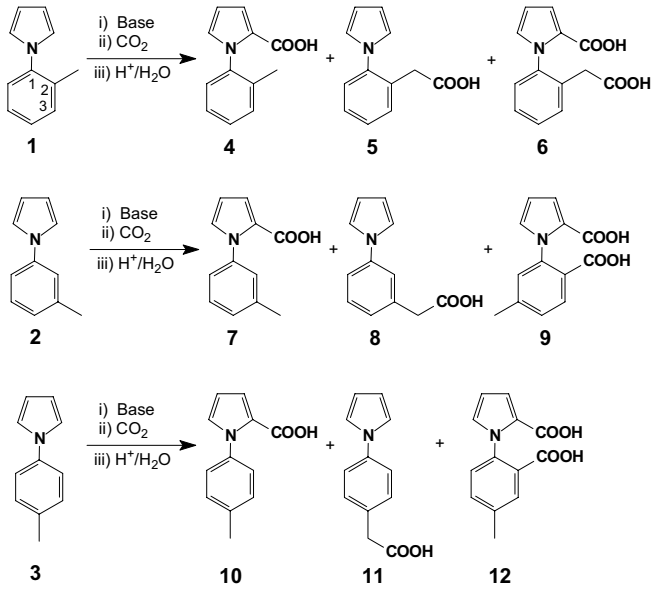

Substituted 1-phenylpyrroles are building blocks for the synthesis of numerous compounds having antibiotic, fungicide, cytostatic effects or CNS activities. In many cases, the standard preparation methods provide these products in low yields because of the sensitivity of the pyrrole ring against acidic conditions. A convenient way for overriding this problem would be the use of organometallic reactions in the synthesis. In order to find efficient methods for optional α *vs*. benzylic metalation as well as dimetalation of the model compounds systematic investigations have been carried out in our laboratory. On the basis of our experimental results novel, regioselective mono- and dimetalation methods have been developed and applied for optional functionalisation of 1-(methylphenyl)pyrroles in α or benzylic or both positions (Scheme).

EXTRACTION OF ST. JOHN'S WORT (*HYPERICUM PERFORATUM* L)

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The proving of St. John's wort curative influence, especially of its antidepressant and antiviral activity, followed its emergence in naturopathic medicine. This brought along a demand for standardized quality extracts. While the efficacy of St. John's wort in depression is now very well established, the mode of action and the constituent(s) responsible for the antidepressant activity have evaded researchers for years. Among the active agents (naphtodianthrones, flavonoids, xanthones, phloroglucinols), several components were found to have antidepressant activity. Conventionally hypericin-standardized extract is made which also contains other active compounds. In the recent years, it appeared that hyperforin had finally been identified as the key constituent responsible for the antidepressant activity of St. John's wort extracts. Hyperforin is labile when exposed to light and air so it cannot be extracted by conventional extraction. Extraction with supercritical carbon dioxide can yield extracts which contain mostly hyperforin, which is stable on prolonged storage. During my work Soxhlet-extraction was carried out with four solvents having various polarity (ethanol 96%, isopropanol, ethyl-acetate, *n*-hexane). Supercritical extraction was studied using carbon dioxide. One of the main active agents, hypericin, was found to concentrate in polar solvents (ethanol, isopropanol). Apolar solvents (*n*-hexane, carbon dioxide) were rich in hyperforin. My further goals are: working out an analytical method for detecting the active components of different extracts, making extracts which contain the most important active agents in their natural form. My aim for the future is preparing standardized extracts of St. John's wort.