These reactions can be very useful for the treatment of synthetic fibres containing these groups. The enzymatic action causes an increase of charged groups at the surface, improving water absorption (increase hydrofility) and dyeability.

Due to enzymatic modification, the acrylic fibers became more hydrophilic and dye uptake was enhanced at temperatures below glass transition. Nitrilase action on PAN fibres was monitored by measuring the release of ammonia and by FTIR detection of the formed of carboxylic groups by diffuse reflectance.

Cutinase were used on polyester fibres. The esterase action on polyester fibres promotes an increase of OH and COOH end groups. The OH end groups can be detected by a titration method and measuring the K/S spectrophotometrically after dyeing with a cotton reactive dye. Similarly enzyme preparations showing amidase activity towards polyamide fibres, promote an increase of NH_2 and COOH end groups. The amine groups were detected by a titration method with an increase of the molecular mass on treated samples. When samples were dyed with a wool reactive dye uptake was enhanced at temperatures below glass transition of polyamide.

P250 NOVEL ENZYME APPLICATIONS ON COTTON CELLULOSE

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Keywords: lipase, protease, hexokinases, cotton fabrics

Lipases and proteases were used to restore partially the strength loss of cotton fabrics, cross-linked with respectively 1,2,3,4-butanetetracarboxylic acids and *N*-hydroxymethyl acryl amide. Nearly one half of the strength loss of the fabrics could be restored by means of enzymatic hydrolysis at low temperature and neutral pH, while the crease-resistance effect decreased only slightly. In another application an enzymatically catalysed phosphorylation of cotton cellulose was achieved using hexokinases in the presence of a phosphate donor adenosine-5'-triphosphate. The enzymatic modification provided a new, reactive type cellulose substrate with improved dyeability and flame-resistance.

P251 LACCASE AND PROTEIN DISULFIDE ISOMERASE ASSISTED DYEING OF KERATINOUS MATERIALS

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Keywords: keratinous fibre, laccase, protein disulfide isomerase

This study reports on the dyeing of keratinous materials using appropriate enzymatic systems – laccases and protein disulfide isomerase. The enzymatic dyeing was performed as a batchwise process at the temperature and pH of maximum enzyme activity. Laccases generate the colour "in situ" starting from low molecular colourless compounds – dye precursor and dye modifiers. Different hues and depth of shades could be achieved varying the concentration of the modifiers and the time of laccase treatment. Protein disulfide isomerases, based on their ability to catalyze thiol-disufide exchange, including oxidation, reduction and rearrangement, were used for covalent fixation of novel cysteine-modified dyes on keratinous fibres.

P252 CHEMO ENZYMATIC PREPARATION OF D-ALLOISOLEUCINE

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Keywords: D-alloisoleucine, subtilisin, diastereoselective hydrolysis

D-Alloisoleucine is a non-proteinogenic amino acid found as a component unit in a number of biologically active depsipeptides, useful intermediates in the synthesis of oxytocin analogues of isostatins and of natural cytotoxic compounds like tamandarins.

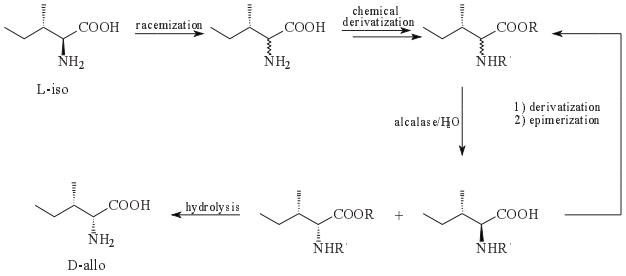
We report here a practical approach to D-alloisoleucine starting from a mixture of L-isoleucine and the D-allo stereoisomer, with an approach both chemical and enzymatic. The latter is based on the use of an industrial hydrolytic enzyme developed for detergency.

Thus L-isoleucine was epimerised and the diastereoisomeric mixture was transformed in a number of *N*-acyl-*O*-es-

ter derivatives including protecting groups usually employed in peptide synthesis.

Hydrolysis in water gave excellent separation of the two diastereoisomers allowing the obtainment of D-alloisoleucine derivatives in the maximum allowable yield in high enantiomeric excess.

During the elaboration of the substrates opportunities to effect the separation of the diastereoisomers *via* crystallization were successfully explored.



P253 PHOSPHOLIPASE D CATALYSED SYNTHESIS OF PHOSPHATIDYLSERINE IN A HOLLOW-FIBER MEMBRANE REACTOR

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Keywords: phospholipase D, hollow fiber membrane, transphosphatidylation

Phospholipase D obtained from a culture of *Streptomyces* sp. PMF, was immobilised in a hollow fiber membrane by ultrafiltration. The reactor was used to contact an organic solution containing phosphatidylcholine (PC, 50 g.l^{-1}) and a water solution at different pH and the rate of formation of the hydrolysis product (phosphatidic acid, PA) was measured. Subsequently the water phase was replaced with a 3M water solution of L-serine and the rate of transphosphatidylation at different pH was evaluated. The reaction was complete in a 48 h period. The formation of the hydrolysis product was minimized working at pH 4.5 were hydrolysis rate is minimal. The operational stability of the system was excellent during a period of several month.

Although the space-time yield of phosphatidylserine formation is lower than in a byphasic CSTR system, the purity of the product and the enzyme consumption is advantageous.

P254 BIOSYNTHESIS OF SESQUITERPENE LACTONES IN CHICORY AND APPLICATION OF THE ENZYMES INVOLVED

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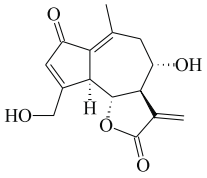
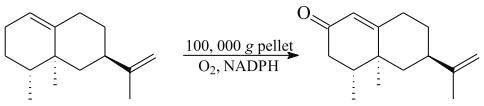
Keywords: sesquiterpenes, biosynthesis, hydroxylation, nootkatone

The edible sprouts of chicory (*Cichorium intybus* L.) are used as a vegetable and are well-known for their slightly bitter taste, which originates from sesquiterpene lactones (*e. g.*, lactucin). Especially the roots contain high concentrations of these bitter principles, which makes them unsuitable for use as cattle feed. However, we have demonstrated that the enzymes responsible for the biosynthesis of the sesquiterpene lactones are still present and active in the roots after the harvest of the sprouts. The first steps in the biosynthetic route have been elucidated by us[1–3].

One of the enzymes involved, the (+)-germacrene A hydroxylase, appears to possess a broad substrate specificity coupled to a high regioselectivity, making this enzyme an attractive catalyst for the hydroxylation of terpenes in flavour and fragrance industry. An especially interesting reaction is the one-step conversion of (+)-valencene into nootkatone[4], a much sought flavour component of grapefruit.



Lactucine



(+)-Valencene Nootkaone