plicated biological samples is thus strictly required for successful approaches to current proteomics.

Hollow-fiber flow field-flow fractionation (HF5) is the innovative, ready-to-market microcolumn version of flow field-flow fractionation (F4) (Zattoni et al., 2007). F4 has been applied as pre-MS step for proteomics (Reschiglian et al., 2008), and current HF5 shows performance comparable to commercial F4. HF5 employs a piece of hollow fiber as separation channel. Low operation flowrates, low dilution of fractionated analytes, and potentially disposable usage to exclude contaminations or carryover make HF5 ideally suited as online or offline pre-MS step for the separation of complex protein samples in native form.

We have developed different applications based on HF5 in combination with MS or LC-MS for the fractionation/purification and characterization of native proteins, and for proteomic analysis of complex biosamples (Reschiglian. et al., 2005),(Zattoni et al., 2008). When applied to the analysis of subproteomes associated with biological nanostructures such as lipoproteins (LPs) in blood serum, HF5 provides fractions corresponding to the different LP classes (Rambaldi et al., 2007) preserving non-covalent interactions between LPs and low-abundance serum proteins (LAP). Preliminary results show HF5 promising for the development of integrated methods to identify not only apo-lipoproteins in the LP classes, but also LP/LAP complexes.

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[P-C.55]

Microbial 1-dehydrogenation of 6-aminomethyl substituted androstenedione

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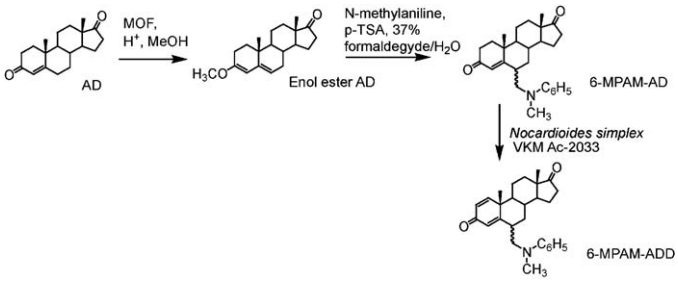
N,N-Disubstituted aminomethylene androstanes, the so called Mannich steroid bases, - are the important structural precursors in the chemical synthesis of 6-methylene- and 6α-methyl-steroids, as well as the perspective substances for the production of novel medical preparations which combine biological activity of the parent compound and potency of the amine constituent (Levina., 1998).

In this work, microbial transformation of 6-aminomethyl substituted steroids by actinobacteria was firstly investigated. Hydrophobic 6-(N-methyl-N-phenylaminomethyl)-androst-4-ene-3,17-dione (6-MPAM-AD) was used as a substrate; *Nocardioides simplex* VKM Ac-2033 was applied as a whole cell biocatalyst for 1-dehydrogenation (Fokina et al., 2003).

The synthesis of 6-MPAM-AD by aminomethylation of androst-4-ene-3,17-dione (AD) in accordance with modified method (Patent and US, 1991) under optimized conditions resulted in the formation of diastereomers mixture (6α- and 6β- isomers) (scheme).



Scheme. Chemical and microbiological steps in the 6-MPAM-ADD combined synthesis from AD.

One isomer of the mixture, namely - 6α-isomer, was found to undergo to biotransformation by *N. simplex* when diastereomers mixtures with different 6α- to 6β-isomer ratio (5/2, 2/1, 1/1 and 1/0.7) were used at the bioconversion.

The solubility of 6-MPAM-AD in aqueous solutions did not exceed 60-70 mg/l, thus resulting in very low bioconversion rate. The enhancement of the reaction and the conversion of both 6α- and 6β-isomers were observed in the presence of methylated β-cyclodextrin (MC): the molar yield of 1-dehydroanalogs of 6α- and 6β-isomers reached 93 and 56%, respectively, as a result of substrate dissolution by complexation with MC (Szejtli, 1991). Neither 1-ene-hydrogenase, nor 17-reducing activity of *N. simplex* was expressed towards 6-MPAM-AD at the conditions used.

The obtaining of 6-(N-methyl-N-phenylaminomethyl)-androsta-1,4-diene-3,17-dione (6-MPAM-ADD) by microbiological means has been hitherto unreported. The structures of 6-MPAM-AD and 6-MPAM-ADD were confirmed using MS, ^{1}H-NMR and element analysis data. The conditions were determined to provide isomerization of 6β-stereomers of 6-MPAM-AD to the more stable 6α-isomers.

The results can be applied at the development of methods for novel aromatase inhibitor obtaining.

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[P-C.56]

An amperometric immunosensor based on nanobiocomposite materials for the determination of alpha-phetoprotein in serum

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Introduction: Immunosensors have become of special interest in several fields, one of their most important applications being the measurement of compounds of clinical or forensic interest in human serum. The present work deals with the development and