B015 Characterization of the protein-core of an arabinogalactanprotein from the roots of *Echinacea pallida* by a new gaschromatographic method

S.Thude, B. Classen, M. Wack and W. Blaschek
Pharmazeutisches Institut der Universität Kiel, Abt. Pharmazeutische Biologie, Gutenbergstr. 76, 24118 Kiel, Germany.

Preparations of roots of *Echinacea pallida* are well known as an herbal immunostimulant. Pharmacological studies have shown that glycoproteins are considered as an active principle (1).
From an aqueous extract of the roots of *E. pallida* we isolated a fraction with a molecular weight of over 30000 Dalton and purified arabinogalactan-type glycoproteins (AGPs) by precipitations with the β-glucosyl Yariv reagent. Structural investigations of this purified AGP revealed a high carbohydrate moiety (about 90%) (2). Also of interest was the qualitative and quantitative composition of the small protein part (<10%). Amino acid composition was analyzed by gas chromatography using a new method (3). After a specific short derivatization-procedure analysis of more than twenty amino acids is possible within about fifteen minutes.

References: 1. Lohmann-Matthes ML and Wagner H (1989) Z. Phytother. 10: 52-59. **2.** Classen B. and Blaschek W. (2000) Carbohydr. Res. 327(4): 497-504. **3.** Phenomenex (2001), EZ: faast® Protein Hydrolysate Kit manual.

B016 Specificity of polyclonal antibodies directed against an arabinogalactan-protein from pressed juice of *Echinacea purpurea*

B. Classen and W. Blaschek
University of Kiel, Institute of Pharmacy, Department of Pharmaceutical Biology, Gutenbergstr. 76, 24118 Kiel, Germany.

Up to now, standardization of Echinacea preparations, which are used as nonspecific immunostimulants, mostly relates to low molecular weight compounds (1). Only once, a method for detection of a polysaccharide/glycoprotein fraction from an extract of *Echinacea purpurea* has been described (2). From pressed juice of *E. purpurea*, we isolated a high molecular weight arabinogalactan-protein (3) with complement stimulating activity (4). With the aim of standardization of Echinacea preparations on AGP, polyclonal antibodies were raised in rabbits.
To characterize the epitope of the antigen, partial acid hydrolysis of the AGP was carried out. The hydrolysis led to loss of arabinose residues at the periphery of the molecule and resulted in loss of reactivity with the antibodies. This reveals that the antibodies are directed against the carbohydrate moiety of the molecule and not against the protein backbone.
To test the specificy of the antibodies, several AGPs from other plants were tested in a Sandwich-ELISA for cross-reactivities. Although there are great similarities in the sugar composition of different AGPs, there were no cross reactivities to AGPs from cell culture of *Echinacea purpurea*, from roots of *E. pallida* and from gum arabic, and only little cross reactivity to an AGP from the aerial parts of *Rudbeckia hirta*. Interestingly, the cross reactivity to an AGP from roots of *Baptisia tinctoria* increased after reduction of the uronic acid residues of this AGP.
There may be two reasons for this specificity of the antibodies:
1) a particular structure of the terminal sugar residues
2) a specific glycosylation pattern, due to a characteristic amino acid sequence of our *Echinacea purpurea* AGP.

References: 1. Bauer, R. in Prendergast, N.L. et al (1998) Plants for food and medicine, Royal Botanic Gardens, Kew. **2.** Egert, D., Beuscher, N. (1992) Planta Med. 58, 163-165. **3.** Classen, B. et al. (2000) Carbohydr. Res. 327, 497-504. **4.** Alban, S. et al., Planta Med., submitted.