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High molecular weight constituents from roots of *Echinacea pallida*: An arabinogalactan-protein and an arabinan

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Abstract

This investigation shows structural features of two macromolecules from roots of *Echinacea pallida* (Nutt.) Nutt: an arabinogalactan-protein (AGP) and an arabinan. The arabinogalactan-protein was precipitated with β-glucosyl Yariv reagent from a high molecular weight fraction. Investigations of the neutral sugar composition revealed Gal (52.1% w/w) and Ara (38.2% w/w) in a ratio of 1.4:1, accompanied by Glc (6.9% w/w) and Rha (2.8% w/w). The content of uronic acids was 6.2%. Mild acid hydrolysis detects Ara and Glc being located at the periphery of the molecule. Linkage analyses and NMR spectroscopy revealed a backbone of the polysaccharide mainly consisting of 3-linked and 3,6-linked Gal*p*-residues. Side chains are composed of 3,6-linked or 6-linked Gal*p* terminating in 5-linked Ara*f*, terminal Ara*f*, Glc*p* and GlcA*p*. The protein part (3.9% w/w) of the AGP is rich in Hyp, Ser, Ala, Thr, Glu, Asp and Gly. The amount of Hyp was determined by a colorimetric method and found to be (0.65% (w/w) of the AGP, which is in good agreement with the result obtained by amino acid hydrolysis (0.67% w/w). The arabinan was isolated from the supernatant of the Yariv precipitation on the basis of solubility in EtOH (80%). It mainly consists of Ara (85.8%). Linkage analyses and NMR spectroscopy indicate a highly branched molecule, consisting of 3,5-linked, 5-linked and terminal Ara*f*-residues in equal amounts.

Keywords: *Echinacea pallida*; Arabinogalactan-protein; Arabinan; Structural analysis

1. Introduction

Preparations of *Echinacea* are well known as herbal immunostimulants, and clinical studies have shown good effects of pressed juice of *Echinacea purpurea* in treatment of the common cold (Hoheisel et al., 1997). Preparations of *Echinacea pallida* (Nutt.) Nutt. are components of many herbal remedies. In vivo studies in mice have proven immunostimulant activities of a high molecular weight extract fraction of the roots of *Echinacea pallida* in combination with other plant extracts by enhancing the antibody response to sheep red blood cells (Bodinet and Freudenstein, 1999) and reducing the pathology of influenza A virus (Bodinet et al., 2002). A stimulation of cytokine titers (TNFα, IL1, IL6) and proliferation of lymphocytes in vitro could be demonstrated by Beuscher et al. (1995). Since many years, different working groups are looking for the pharmacological active compounds of *Echinacea* species. Besides caffeic acid derivatives and alkamides, polysaccharides and glycoproteins are supposed to be active principles (Bauer, 1998). Especially polysaccharides and glycoproteins containing high amounts of arabinose and galactose are often considered to be important (e.g. Beuscher et al., 1995; Lohmann-Matthes and Wagner, 1989; Wagner et al., 1989).

Arabinogalactan-proteins contain a high carbohydrate (>90%) and a small protein (<10%) moiety. They have been isolated from cell walls and exudates of lower and higher plants (for review see Nothnagel, 1997) as

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well as from plant suspension cultures (Sims and Bacic, 1995; Zhu et al., 1993). In plants, AGPs are known to have different functions, e.g., they induce somatic embryogenesis (Kreuger and van Holst, 1993), control cell proliferation and expansion (Van Hengel et al., 2002) and are involved in programmed cell death (Gao and Showalter, 1999). Looking at their pharmacological potential, there are interactions of AGPs with the complement system in vitro (Alban et al., 2002; Diallo et al., 2001).

Highly branched arabinans have been isolated from leaves, seeds, vegetables and fruits (Siddiqui and Emery, 1990; Kraus, 1991; Swamy and Salimath, 1991). They are generally associated with polysaccharides of the cell wall, either as homoglycans or as part of crosslinked pectins, more precisely as side chains of rhamnogalacturonans (Aspinall, 1973). Little is known about their function. In addition to the contribution of arabinans to cell wall structure, Jones et al. (2003) have demonstrated their importance for the regulation of guard cell function. Pharmacologically there are indications of anti-complementary activities of arabinans in vitro (Yamada et al., 1988; Zhao et al., 1994). This paper for the first time shows the isolation and structural characterization of an AGP and an arabinan from dried roots of *Echinacea pallida*, which may be candidates for immunomodulatory activity.

2. Results and discussion

2.1. Isolation, yield and molecular weight of AGP and arabinan

Free proteins of the extract were removed by centrifugation after 10 min of boiling. Components with a molecular weight <30,000 Da like alkamides, fructans and others were cut off by two steps of dialysis. The yield of the high molecular weight fraction (>30,000 Da) were between 0.2% and 0.6% of dry weight of the plant material. From this fraction, AGP was precipitated with Yariv reagent (yield: 6.0–12.9% of the freeze-dried high molecular weight fraction, 0.01–0.08% of dry weight of the roots). Size exclusion chromatography reveals a single fraction as a narrow, nearly symmetrical peak with a hydrodynamic volume of 1.1×10^6 Da. The determined molecular weight is comparable with that of an AGP from *Echinacea purpurea* (1.2×10^6 Da) (Classen et al., 2000). The arabinan was isolated from the supernatant of the Yariv precipitation according to its solubility in EtOH 80%. In contrast to most other polysaccharides, arabinans are not precipitated by EtOH 80% (Siddiqui and Emery, 1990; Blaschek, 1991). Determination of the hydrodynamic volume by size exclusion chromatography reveals a single symmetrical peak. Compared to pullulans with known molecular weight, this fraction has an estimated molecular weight of 9.7×10^5 Da. The yield of the arabinan from the supernatant was between 4.6% and 11.7% of the high molecular weight fraction and 0.01–0.07% of dry weight of the roots.

2.2. Composition of the AGP – protein moiety

Determination of the amino acids of the AGP by HPLC and ninhydrine post column derivatisation resulted in a protein part of 3.9% (w/w) calculated from the amino acid analysis. The protein moiety is characterized by high amounts of Hyp (17.3%), Ser (11.4%), Ala (9.6%), Thr (8.9%), Glu (8.6%), Asp (7.3%) and Gly (7.0%). Minor components were Val (5.6%), Lys (4.2%), Leu (3.9%) and Pro (3.4%). Traces of Isoleu, Met, Phe, His, Tyr and Arg were also found. The amount of Hyp has also been determined by a photometric method, which revealed a concentration of 0.65% Hyp per AGP (w/w). This is in good agreement with the amino acid analysis (0.67% Hyp per AGP (w/w)). The composition of the protein part, especially high amounts of Hyp, Ser, Thr and Ala, is typical for AGPs. Most likely the carbohydrate moieties are *O*-glycosidically linked to Hyp residues of the protein part (Bacic et al., 2000).

2.3. Composition of the AGP – carbohydrate moiety

Neutral monosaccharides were determined after hydrolysis, reduction and acetylation, followed by GC. The AGP revealed a sugar composition of Gal (52.1% w/w) and Ara (38.2% w/w) in a ratio of 1.4:1. Minor components were Glc (6.9% w/w) and Rha (2.8% w/w). The content of uronic acids was measured photometrically (Blumenkrantz and Asboe-Hansen, 1973) and determined to be 6.2% (w/w) of the AGP.

2.4. Linkage analyses of the carbohydrate moiety of AGP

AGP was subjected to linkage analyses by the method of Harris et al. (1984) with and without previous reduction of carboxyl groups (Table 1). Ara was found to be in the furanose form, the major part of it was terminally linked, but also high amounts of 5-linked Ara*f* could be detected. 6-linked Gal, terminal Gal, Glc and Rha were present in the pyranose form. The high amount of 3,6-linked Gal*p* and missing of 4-linked Gal*p* points to a type II arabinogalactan according to the classification of Aspinall (1973).

Uronic acids were detected after reduction, methylation analysis and GC-MS as their corresponding neutral monosaccharides and found to be terminally linked GlcA*p*. The results (Table 1) also allow a proposal concerning the position of terminal GlcA. Without reduction of uronic acids, hydrolysis of the polysaccharide is incomplete, resulting in formation of aldobiuronic

Table 1
Linkage types of the polysaccharide moiety of AGP

Sugar residue	Linkage	AGP (without reduction of uronic acids), mol%	AGP (with reduction of uronic acids), mol%
Ara*f*	Terminal	29.4	31.4
	5-	14.2	13.1
Gal*p*	Terminal	2.5	2.4
	3-	16.5	11.1
	6-	6.3	9.2
	3,6-	26.9	24.8
Glc*p*	Terminal	4.2	2.9
GlcA*p*	Terminal	n.d.	5.0
Rha*p*	Terminal	tr.	tr.

acids and missing detection of uronic acids and neighbouring linked neutral monosaccharides. The only neutral monosaccharide which increases after reduction of uronic acids is 6-linked Gal, pointing to a 6-linked Gal carrying terminal GlcA at position 6.

2.5. Partial acid hydrolysis

The AGP was partially hydrolyzed by oxalic acid and precipitated with EtOH, resulting in a high molecular weight moiety (GP) being insoluble in EtOH, and a low molecular weight supernatant composed of mono- and oligosaccharides (A). The composition of AGP and both fractions after mild hydrolysis is shown in Table 2. Most of the Ara-bonds had been cleaved, whereas the Gal-bonds remained more or less stable. The ethanol insoluble "core" polysaccharide (GP) was composed of mainly Gal, whereas the supernatant mainly contained Ara, suggesting that Ara is located at the periphery of the AGP, whereas Gal is located in the center of the polysaccharide part. The high amount of Glc in the supernatant shows, that Glc is also located at the periphery and possibly linked to Ara. The supernatant of the precipitation with EtOH was investigated without (A-mono) and with TFA-hydrolysis (A) prior to acetylation and GC. Without TFA hydrolysis, only monosaccharides present in A are found, whereas with TFA hydrolysis also monosacharides released from oligosaccharides are detected. As in A compared to A-mono much more Gal-residues are found (Table 2), most of the Gal residues in the supernatant are present as di- or oligosaccharides.

The fractions GP and A have also been subjected to methylation analyses (Table 3). Comparing AGP and the polysaccharide "core" (GP), the content of 3-linked Gal is nearly equal in AGP and GP. Furthermore there is nearly a complete loss of Ara, a high increase of terminal and 6-linked Gal and a decrease of 3,6-linked Gal in GP. Therefore most of the Ara is linked to 3,6-Gal and not to 3-Gal. The supernatant mainly consists of terminal Ara*f* and Glc*p*. There are still 7% of 5-linked Ara in the supernatant, showing that oxalic acid hydrolysis did not decompose all Ara bonds.

In order to specify the location of the uronic acid, the "core" polysaccharide (GP) was reduced (Taylor and Conrad, 1972) and methylated (Table 3, GP-red.). Terminal GlcA*p* was found, confirming that bonds to GlcA are not cleaved by mild hydrolysis and GlcA is linked to Gal and not to Ara.

2.6. ^{13}C NMR spectroscopy of AGP

Peaks were assigned by comparison with published spectra (Classen et al., 2000; Gane et al., 1995; Tan et al., 2004). The spectrum (Table 4a) shows two sharp signals of C-1 of terminal α-L-Ara*f* (108.7 ppm) and 5-linked Ara*f* (106.9 ppm). C-1 of 3-, 6- and 3,6-linked β-D-Gal*p* appear as a cluster of signals at about 103.1 ppm. There are no signals relating to α-glycosidic linkages of Gal. Characteristic responses of ring carbons C-2–C-4 of Ara are between 76.0 and 83.4 ppm, C-5 unsubstituted at 60.3 ppm and substituted at 66.1 ppm. The signals of Ara are much sharper than those of Gal. This indicates, that Ara has an enhanced mobility

Table 2
Composition of neutral monosaccharides of AGP and its degradation products after partial acid hydrolysis

Monosaccharide (weight%)	AGP (Yariv precipitate)	GP (EtOH 80% insoluble fraction after partial hydrolysis)	A (supernatant of the EtOH soluble fraction after partial hydrolysis)	A-mono (supernatant of the EtOH soluble fraction after partial hydrolysis without TFA-hydrolysis)
Ara	38.2	5.1	69.4	82.6
Gal	52.1	94.9	16.1	2.1
Glc	6.9	–	9.0	11.5
Other	2.8	–	5.5	3.8

Table 3
Linkage analysis of AGP and the degradation products after partial acid hydrolysis and reduction of uronic acids

Sugar residue	Linkage	AGP, mol%	GP, mol%	A, mol%	GP-red (after reduction of uronic acids), mol%
Ara*f*	Terminal	29.4	3.5	61.7[a]	2.1
	5-	14.2	–	6.8	–
Gal*p*	Terminal	2.5	17.6	–	10.8
	3-	16.5	17.8	–	14.8
	6-	6.3	43.5	11.4	42.7
	3,6-	26.9	17.2	–	16.1
Glc*p*	Terminal	4.2	0.4	20.1	1.4
GlcA*p*	Terminal	ad.	n.d.	ad	12.1
Rhap	Terminal	tr.	–	tr.	–

[a] Ara*f* = 33.7%; Ara*p* = 28.0%.

Table 4a
Summary of ^{13}C NMR chemical shifts for AGP

Glycosidic linkage	Chemical shifts (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
Terminal α-L-Ara	108.7	80.7	76.0	83.4	60.3	
5-α-L-Ara	106.9	80.3	76.0	81.8	66.1	
3-β-D-Gal	103.1	69.7	80.9	68.0	74.3	60.6
6-β-D-Gal	103.1	70.2	72.3	68.0	72.9	68.6
3,6-β-D-Gal	103.1	69.4	80.9	68.0	72.9	68.6

Table 4b
Summary of ^{13}C NMR chemical shifts for arabinan

Glycosidic linkage	Chemical shifts (ppm)				
	C-1	C-2	C-3	C-4	C-5
Terminal α-L-Ara	107.4	81.2	76.5	83.8	61.0
5-α-L-Ara	107.4	80.8	76.5	82.2	66.2
3, 5-α-L-Ara	107.0	81.2	79.1	82.2	65.8

consistent with the location at the periphery of the molecule, whereas Gal forms the backbone inside the molecule, which is in good agreement with the data obtained by methylation analysis.

2.7. Structural proposal

Combining the results of structural investigations, the AGP consists of a backbone of 3-linked and 3,6-linked Gal. The side chains are made up of 6-linked Gal and 3,6-linked Gal, terminating in GlcA. Furthermore side chains are branched at position 3 to 5-linked Ara, associated with terminal Ara or Glc. The proposed structure of the carbohydrate moiety is consistent with models of arabino-3,6-galactans type II (Aspinall, 1973).

Compared to an AGP isolated from pressed juice of *Echinacea purpurea*, both structures show similarities, the ratio of Aral/Gal (*Echinacea pallida* 1/1.4; *Echinacea purpurea* 1/1.8) being slightly different, mainly due to a higher content of 6-Gal in the AGP from *Echinacea purpurea*.

2.8. Composition of arabinan

The arabinan mainly consists of Ara (85.8%) accompanied by some Glc (7.7%), Rha (3.9%) and traces of Gal and Man (together 2.6%).

The results after methylation analysis and GC-MS indicated three linkage-types of Ara in the furanose form: terminal Ara (34.0 mol%), 5-linked Ara (28.4 mol%) and 3,5-linked Ara (33.3 mol%). Traces of terminal Glc (2.0 mol%) and terminal Rha (2.3 mol%) in the pyranose form could also be detected. The linkage types suggest a highly branched polysaccharide, quite similar to other arabinans, which show the same type of linkage, but different molar ratios of Ara residues (Stevens and Selvendran, 1980; Siddiqui and Emery, 1990).

2.9. ^{13}C NMR spectroscopy of arabinan

Identification of signals was done on the basis of published data (Navarro et al., 2002; Swamy and Salimath, 1991) and methylation analysis. Table 4b shows, that

all anomeric signals (C-1) at 107.0 or 107.4 ppm are from α-L-Ara*f*, because shifts of β-L-Ara*f* would be in the higher field (about 102.7–105.1 ppm). Characteristic signals for ring carbons C-2 and C-4 are detected between 76.5 and 83.8 ppm. Signal of substituted C3 has moved downfield (79.1 ppm; unsubstituted 76.5 ppm), while C4 of all sugar residues are quite similar (82.2 or 83.8 ppm). The signal for unsubstituted C5 is at 61.0 ppm and for substituted C5 in lower field at 65.8 or 66.2 ppm. Altogether data of NMR-spectroscopy confirm results of methylation analysis.

3. Experimental

3.1. Material

Dried roots of *Echinacea pallida* (Nutt.) Nutt. have been submitted by Alfred Galke GmbH, Gittelde, Germany.

3.2. Synthesis of the β-glucosyl Yariv reagent

The reagent (1,3,5-tris[4-β-D-glucopyranosol-oxyphenylazo]-2,4,6-trihydroxybenzene) was prepared as described by Yariv et al. (1962).

3.3. Isolation of arabinan and AGP

Drug material (400 g) was powdered and macerated for 16 h in 2 l of aqua dem. The extract was centrifuged (5000*g*, 10 min) and the supernatant boiled for 10 min. After centrifugation (5000*g*, 10 min) the supernatant was dialysed by tangential cross flow filtration (Biomax membrane, MWCO 30 kDa, Millipore, Eschborn, Germany). The fraction larger than 30 kDa was purified by dialysis (molecularporous membrane tubing, 12–14 kDa, Spectra/Por, Houston, USA). The freeze-dried high molecular weight fraction was dissolved in distilled water (10 mg/ml) and precipitated with the same volume of an aqueous solution of β-glucosyl Yariv reagent (1 mg/ml). After addition of NaCl up to a final concentration of 0.15 M (Kreuger and van Holst, 1993) the AGP-Yariv complex was precipitated at 4 °C overnight, centrifuged (10,000*g*, 15 min) and redissolved in distilled water. The complex was heated to 50 °C and sodium hydrosulfite ($Na_2S_2O_4$) was added (about 10% (w/v)) until the red colour disappeared and the Yariv-AGP complex (Kreuger and van Holst, 1995) was destroyed. The light amber solution was dialysed for four days against water at 4 °C and freeze-dried (AGP). The supernatant of the AGP-Yariv complex was treated in the same way. The freeze dried supernatant was redissolved in water and EtOH added up to a concentration of 80% (v/v), stored at 4 °C overnight and centrifuged (10,000*g*, 10 min). The supernatant was evaporated, redissolved in water and freeze-dried (arabinan).

3.4. Size exclusion chromatography

One mg freeze dried substance was dissolved in 1 ml NaCl solution (1 g/100 ml), filtered (0.45 μm), applied to a column of Sephacryl-S400 (Pharmacia, Freiburg, Germany) and the eluent monitored by mini Dawn, a Laser Light Scattering Instrument (Wyatt, Santa Barbara, USA). Molecular weights of AGP and arabinan were determined using pullulans (Shodex, Denkko, Japan) with known masses as reference polysaccharides.

3.5. Analysis of sugar components

After hydrolysis (TFA 2 M, 1 h, 121 °C) the neutral monosaccharides were reduced with sodium borohydride and acetylated with Ac_2O. The alditole acetates were investigated by GC (Blakeney et al., 1983).

3.6. Determination of amount of uronic acids

Colorimetric determination of uronic acids was performed after hydrolysis (sulfuric acid, 1 h, 121 °C) and reaction with 3-hydroxydiphenyl (Blumenkrantz and Asboe-Hansen, 1973).

3.7. Reduction of carboxyl groups

The carboxyl groups of uronic acids were activated with 1-cyclohexyl-3-[2-methylmorpholinoethyl]-carbodiimide-metho-4-toluolsulfonate and reduced with sodium borodeuteride (Taylor and Conrad, 1972).

3.8. Methylation analysis

Methylation was accomplished with potassium methylsulfinyl carbanion and methyl jodide followed by hydrolysis and acetylation (Harris et al., 1984). GC–MS-analyses of partially methylated alditole acetates were performed on a fused silica capillary column (0.25 i.d. × 25 m, OV-1701, Macherey & Nagel, Düren, Germany; temperature program: 3 min 120 °C, 8 °C/min up to 170 °C, 1 °C/min up to 200 °C, 70 °C/min up to 240 °C, hold for 3 min). Helium flow was 1 ml/min. Mass spectra were recorded on a HP MS Engine 5898A (Hewlett–Packard) instrument (ionization potential 70 eV; source temperature 200 °C).

3.9. Partial acid hydrolysis

Oxalic acid (12.5 mM, 0.1 ml/mg AGP) was used for partial acid hydrolysis at 100 °C for 5 h (Gleeson and

Clarke, 1979). The hydrolysate was dissolved in EtOH up to a final concentration of 80% (v/v), stored at 4 °C overnight and centrifuged (20,000*g*, 10 min). After two washing steps of the precipitate with EtOH 80% the supernatant combined with the washing solutions (A) and the purified precipitate (GP) were freeze-dried.

3.10. Composition of amino acids

AGP was hydrolysed at 110 °C for 24 h. Composition of amino acids was investigated by HPLC with post column ninhydrine derivatisation (AminoSys, now: AminoNova, Berlin, Germany). Absorption was measured at 440 and 570 nm.

3.11. Determination of hydroxyproline

AGP was hydrolysed at 110 °C for 24 h. After oxidation with sodium-*N*-chloro-*p*-toluolsulfonamide and addition of a mixture of 4-dimethylaminobenzaldehyde, perchloric acid and isopropanol, the specific absorption was measured (558 nm). Solutions containing rising amounts of hydroxyproline were used as standards (Lebensmittel- und Bedarfsgegenständegesetz, LMBG, §35, L06.00/8).

3.12. NMR spectroscopy

Samples of AGP and arabinan were dissolved in D_2O (about 20 mg/ml) and ^{13}C NMR spectra were recorded with ^{1}H composite pulse decoupling on a Bruker ARX-300 spectrometer at 75.5 MHz at 27 °C for 18 h with a 2 s pulse delay, 2.2 s acquisition time and 90° pulse angle. Chemical shifts were set relative to dioxane (66.5 ppm).

References

Alban, S., Classen, B., Brunner, G., Blaschek, W., 2002. Differentiation between the complement modulating effects of an arabinogalactan- protein from *Echinacea purpurea* and heparin. Planta Med. 12, 1118–1124.

Aspinall, G.O., 1973. Carbohydrate polymers of plant cell walls. In: Loewus, F. (Ed.), Biogenesis of Plant Cell Wall Polysaccharides. Academic Press, New York, London, p. 95.

Bacic, A., Currie, G., Gilson, P., Mau, S.L., Oxley, D., Schultz, C.J., Sommer-Knudsen, J., Clarke, A.E., 2000. Structural classes of arabinogalactan-proteins. In: Nothnagel, E.A., Bacic, A., Clarke, A.E. (Eds.), Cell and Developmental Biology of Arabinogalactan-Proteins. Kluwer Academic Publishers/Plenum, Dordrecht, Netherlands/New York, pp. 11–23.

Bauer, R., 1998. The Echinacea story – the scientific development of an herbal immunostimulant. In: Prendergast, H.D., Etkin, N.L., Harris, D.R., Houghton, P.J. (Eds.), Plants for Food and Medicine. Royal Botanic Gardens, Kew, pp. 317–332.

Beuscher, N., Bodinet, C., Willigmann, I., Egert, D., 1995. Immunmodulierende Eigenschaften von Wurzelextrakten verschiedener *Echinacea*-Arten. Z. Phytother. 16, 157–166.

Blaschek, W., 1991. Isolierung und Analytik von Polysacchariden. In: Franz, G. (Ed.), Polysaccharide. Springer-Verlag, New York, pp. 16–48.

Blakeney, A.B., Harris, P.J., Henry, R.J., Stone, B.A., 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohydr. Res. 113, 291–299.

Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54, 484–489.

Bodinet, C., Freudenstein, J., 1999. Effects of an orally applied aqueous- ethanolic extract of a mixture *of Thujae occidentalis* herba, *Baptisiae tinctoriae* radix, *Echinaceae purpureae* radix and *Echinaceae pallidae* radix against sheep red blood cells in mice. Planta Med. 65, 695–699.

Bodinet, C., Mentel, R., Wegner, U., Lindequist, U., Teuscher, E., Freudenstein J, 2002. Effect of oral application of an immunomodulating plant extract on Influenza virus type A infection in mice. Planta Med. 10, 896–900.

Classen, B., Witthohn, K., Blaschek, W., 2000. Characterization of an arabinogalactan-protein isolated from pressed juice of *Echinacea purpurea* by precipitation with the β-glucosyl Yariv reagent. Carbohydr. Res. 327, 497–504.

Diallo, D., Paulsen, B.S., Liljeback, T.H., Michaelsen, T.E., 2001. Polysaccharides from the roots of *Entada Africana* Guill. et Perr., Mimosaceae with complement fixing activity. J. Ethnopharmacol. 74, 159–171.

Gao, M., Showalter, A.M., 1999. Yariv reagent treatment induces programmed cell death in *Arabidopsis* cell culture and implicates arabinogalactan-protein involvement. Plant J. 19, 321–331.

Gane, A.M., Craik, D., Munro, S.L., Howlett, G.J., Clarke, A.E., Bacic, A., 1995. Structural analysis of the carbohydrate moiety of arabinogalactan-proteins from stigmas and styles of *Nicotiana alata*. Carbohydr. Res. 277, 67–85.

Gleeson, P.A., Clarke, A.E., 1979. Structural studies on the major component of *Gladiolus* style mucilage, an arabinogalactan-protein. Biochem. J. 181, 607–621.

Harris, P.J., Henry, R.J., Blakeney, A.B., Stone, B.A., 1984. An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. Carbohydr. Res. 127, 59–73.

Hoheisel, O., Sandberg, M., Bertram, S, Bulitta, M., Schäfer, M., 1997. Echinagard treatment shortens the course of the common cold: a double- blind, placebo-controlled clinical trial. Eur. J. Clin. Res. 9, 261–268.

Jones, L., Milne, J.L., Ashford, D., McQueen-Mason, J., 2003. Cell wall arabinan is essential for guard cell function. Proc. Natl. Acad. Sci. USA 100, 11783–11788.

Kraus, J., 1991. Water-soluble polysaccharides from *Ginkgo biloba* leaves. Phytochemistry 30, 3017–3020.

Kreuger, M., van Holst, G.J., 1993. Arabinogalactan-proteins are essential in somatic embryogenesis of *Daucus carota* L. Planta 189, 243–248.

Kreuger, M., van Holst, G.J., 1995. Arabinogalactan-protein eptitopes in somatic embryogenesis of *Daucus carota* L. Planta 197, 135–141.

Tan, L., Qiu, F., Lamport, D.T., Kieliszewski, M.J., 2004. Structure of a hydroxyproline (hyp)-arabinogalactan polysaccharide from repetitive ala-hyp expressed in transgenic *Nicotiana tabacum*. J. Biol. Chem. 279, 13156–13165.

Lohmann-Matthes, M.L., Wagner, H., 1989. Aktivierung von Makrophagen durch Polysaccharide aus Gewebekulturen von *Echinacea purpurea*. Z. Phytother. 10, 52–59.

Navarro, A.D., Cerezo, A.S., Stortz, C.A., 2002. NMR spectroscopy and chemical studies of an arabinan-rich system from the endosperm of the seed of *Gleditsia triacanthos*. Carbohydr. Res. 337, 255–263.

Nothnagel, E.A., 1997. Proteoglycans and related components in plant cells. In: Jeon, K.W. (Ed.), International Review of

Cytology – A Survey of Cell Biology. Academic Press, San Diego, pp. 195–291.

Siddiqui, I.R., Emery, J.P., 1990. Studies on vegetables. Investigation of an arabinan from parsnik (*Pastinica sativa*). J. Agric. Food Chem. 38, 387–389.

Sims, J.M., Bacic, A., 1995. Extracellular polysaccharides from suspension cultures of *Nicotina plumbaginifolia*. Phytochemistry 38, 1397–1405.

Stevens, B.J., Selvendran, R.R., 1980. Structural investigation of an arabinan from cabbage (*Brassica oleracea* var. *capitata*). Phytochemistry 19, 559–561.

Swamy, N.R., Salimath, P.V., 1991. Arabinans from *Cajanus cajan* cotyledon. Phytochemistry 30, 263–265.

Taylor, R.L., Conrad, H.E., 1972. Stoichiometric depolymerisation of polyuronides and glycosamino-glycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. Biochemistry 11, 1383–1388.

Wagner, H., Stuppner, H., Puhlmann, J., Brümmer, B., Deppe, K., Zenk, M.H., 1989. Gewinnung von immunologisch aktiven Polysacchariden aus *Echinacea*-Drogen und -Gewebekulturen. Z. Phytother. 10, 35–38.

Van Hengel, A.J., van Kammen, A., de Vries, S.C., 2002. A relationship between seed development, arabinogalactan-proteins (AGPs) and the AGP mediated promotion of somatic embryogenesis. Physiol. Plant. 114, 637–644.

Yamada, H., Ra, K.S., Kiyohara, H., Cyong, J.C, Otsuka, Y., 1988. Characterization of anti-complementary neutral polysaccharides from the roots of *Bupleurum falcatum*. Phytochemistry 27, 3163–3168.

Yariv, J., Rapport, M.M., Graf, L., 1962. The interaction of glycosides and saccharides with antibody to the corresponding phenylazo glycosides. Biochem. J. 85, 383–388.

Zhao, Q.C., Kiyohara, H., Yamada, H., 1994. Anti-complementary neutral polysaccharides from leaves of *Artemisia* princes. Phytochemistry 35, 73–77.

Zhu, J.K., Bressan, R.A., Hasegawa, P.M., 1993. Loss of arabinogalactan-proteins from the plasma membrane of NaCl-adapted tobacco cells. Planta 190, 221–226.