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Bioactive Pectic Polysaccharides

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Abstract Polysaccharides from plants have been the subject of studies for a very long time, mainly focussed on their physical properties, their chemical and physical modification, and their application. Over the last 20 years there has been increasing interest

in the biological activity of the natural polysaccharide polymers. These studies became possible as a result of the scientific development of isolation, purification and characterisation methods concomitant with the development of fairly simple in vitro tests for effects especially on the immune system. The growing acceptance of the knowledge to be gained by people still using so-called traditional medicine in finding sources worthy of study has led to new sources for interesting bioactive plant polysaccharides. This chapter contains only the knowledge on bioactive plant polysaccharides of the pectic type gained over approximately the last ten years and the focus is on those papers where structural characterisation has been performed. For this reason, the reader may not find all of the plants studied during this period within the chapter. Discussions concerning the structural aspects of polysaccharides that may be responsible for activity, are included where relevant.

Keywords Arabinogalactans · Bioactivity · Medicinal plants · Rhamnogalacturonans · Pectins · Structure-activity relations

Abbreviations

AceA	aceric acid
AFM	atomic force microscopy
AG-I	arabinogalactan type I
AG-II	arabinogalactan type II
Api	apiose
Ara*f*	arabinofuranose
DHA	3-deoxy-D-lyxo-2-heptulosaric acid
DP	Degree of polymerisation
Gal*p*	galactopyranose
Gal*p*A	galactopyruronic acid
GlcA	glucuronic acid
Glc*p*	glucopyranose
IL-6	interleucine 6
KDO	3-deoxy-D-manno-octulosonic acid
NK cells	natural killer cells
p.o.	per os
RG-I	rhamnogalacturonan type I
RG-II	rhamnogalacturonan type II
Rha*p*	rhamnopyranose
Xyl	xylose

1 Introduction

In traditional medicine, plants have been used to treat various types of illnesses, including wounds, both external and internal. The use of plants can be found as part of traditional medicine on all continents, and plants are still in use even in the Western countries as so-called “traditional remedies”. Modern science has shown that many of these plants contain polysaccharides

that exhibit biological activity of different kinds. In addition, many of these polysaccharides are able to form gels or viscous solutions that are of great industrial value. Research to form a scientific basis for rational, traditional use of polysaccharides isolated from plants as immunostimulatory, antitumour or anti-inflammatory agents was not possible before approximately 20 years ago. This was according to Wagner and Kraus [1] mainly due to following reasons:

- The isolation and purification of sufficient amounts of pure bioactive polysaccharides in a reproducible manner was difficult due to the lack of isolation and purification methods.
- Unknown structure activity relationships, mainly because methods for activity testing were poorly available.
- Lack of information concerning the exact mechanism of the action for the immunomodulatory pharmacological activities.
- Lack of information concerning pharmacokinetics and bioavailability after p.o. and parenteral administration of the polymers.

The groups of Wagner in München, Germany, and Yamada in Tokyo, Japan, have made important contributions towards the development of methods that could be used for testing the biological activity of polysaccharides in vitro and in vivo, which are well documented in various reviews in the field [2–7]. Yamada [4] reports that 1984 was the first year when studies on a pure, complex polysaccharide with biological activity were cited in the literature. This was isolated from the upper part of *Echinacea purpurea*, a plant that had a long traditional use against cold and influenza. The use of this plant was first reported from the Native Americans of the old North America, and another traditional use was as a remedy for woundhealing [8]. Soon after this report, further studies of bioactive polysaccharides were published and most of the studies performed on biologically active polysaccharides took the traditional information on the use of plants in woundhealing or related areas as the starting point for choosing what plants were to be studied. Especially plants used for the treatment of external wounds and dermal ailments, as well as those used against ulcer and tumours, were early in focus.

Plants, lichens and algae have all been tested for the content of polysaccharides with biological effect in various systems. Polysaccharides appear in many different forms and in different locations in plants. They are present in all organs, both inside cells, and intercellularly, and they are important as strengthening substances, i.e. as fibres and as the material forming the matrix of the cells. Polysaccharides are structurally a heterogeneous group of compounds, they are neutral or acidic, they may consist of only one type of monosaccharide, or of two and up to approximately ten different types, some of which may be in repeating units; they can be linear or branched and be substituted with different types of organic groups like methyl and acetyl groups. Often the polysaccharides with biological activity are charged, that is they contain uronic acids, e.g. D-galacturonic acid as in the pectic

type polymers. Different types of polysaccharides isolated from plants used in traditional medicine are identified for their activities on the complement system; e.g. arabinans, arabinogalactans and rhamnogalacturonans [4]. Similar types of polymers have also been shown to have effects on macrophages, T-lymphocytes and NK-cells. The majority of these polysaccharides exhibit also a mucoadhesive effect. They bind to the surface of cells, and can then be the cause of local effects seen in certain experiments [9].

This review will focus on pectic substances that have been shown to exhibit biological activity. Their structure, bioactivity and possible structure activity relations will be discussed.

2
Chemical Structure of Pectic Type Polymers

Pectins can generally be divided into neutral and acidic polymers, but certain structural features are common between the different types of pectic substances. These will be described below. Concerning the general chemistry of carbohydrate types of linkages, the reader can find details on this in general textbooks of chemistry and biochemistry and is for this reason not included.

2.1
Arabinans

The arabinans found in plants are basically composed of L-arabinofuranosides. Depending on the source they may be linear or branched, and primarily linked through positions 3 or 5, Fig. 1 [10, 11]. Linkages through C-2 are also observed, but generally they are less frequent than the 3-linkage. It is generally accepted that the core linkage of the arabinans are the 5-linkages and the branches occur at C-3 or C-2. But it is not obvious that the arabinans exist as such in nature. They are most probably linked to the galactans in the pectic complex and released either via enzymatic action or weak acid hydrolysis during the extraction process. The cell-walls where the arabinans are found are also rich both in *exo*- and *endo*-glycanases.

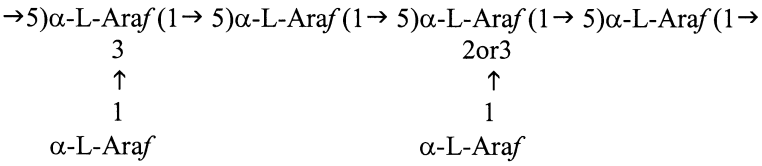

Fig. 1 Proposed structure of a part of an arabinan

A few examples of pure arabinans with effect in the complement system have been isolated from, for example, the fruits of *Ziziphus jujuba* (α-2,5-arabinofuranan) and from the roots of *Bupleurum falcatum* (α-3,5-arabinofuranan-α-1,4-glucan complex) [12, 13].

2.2 Arabinogalactans Type I and II (AG-I and AG-II)

The arabinogalactans have more frequently been reported for activity in various biological systems. Arabinogalactans are often classified in three groups: arabino-4-galactans (Type I), arabino-3,6-galactans (Type II) and polysaccharides with arabinogalactan side chains (Type III) [14]. The latter type are also called the real pectins [10, 11]. Only types I and II will be dealt with in this chapter, as Type III are equal to the pectins discussed below.

AG-I is found to variable degrees in the cell wall and is composed of a β-1,4 linked galactan backbone with side chains of arabinans basically linked through position 3 of the galactose units. The AG-I structures are mainly found as a constituent of the pectic complex RG-I that will be described below. AG-II has as its main core a galactan that can have either 3 or 6 linkages in the main chain and is highly branched with the 1,3,6-linked galactose units at the branching points. Also this type of arabinogalactan is frequently found bound to RG-I. Both types of arabinogalactans are found to be linked through position 4 of the rhamnose units of the pectic chain (see below). One easy method to distinguish between the two arabinogalactans is their ability to precipitate the so called Yariv reagent [15, 16]. Only AG-II has the ability to form a red precipitate with the Yariv reagent, and this is frequently used to show the presence of AG-II in bioactive polymers, and can also be used for a quantitative assessment of the amount of this type of polymer in the total pectic complex [6].

2.3 Pectic Acid, Pectins

Pectic acids or pectins are common words for describing polymers containing galacturonic acid. These were earlier thought to mainly consist of the acid only, but it has now long been recognised that pectins are a very complex group of polysaccharides. Long sequences of polygalacturonans (homogalacturonans) can be found in the pectins, and it has been shown by, for example, Nothnagel et al. [17] and Samuelsen et al. [18] that the galacturonan part of the pectins may consist of up to at least ten units. Several neutral monosaccharides are normally also present in these polymers. Pectins are found mainly between the cells and in the primary cell wall in most plants [10, 11], and have during the last 20 years been shown to be responsible for different types of bioactivity when used as traditional medicines in differ-

ent cultures [1–4, 19]. The pectic type of polymer possess complex structures, as mentioned, and today they are divided into two main types, Rhamnogalacturonan I and Rhamnogalacturonan II. They differ so much in structure that it is feasible to deal with them separately [10].

2.4 Rhamnogalacturonan I (RhaGalA-I, RG-I)

Rhamnogalacturonan I or RG-I was first used by Albersheim's group [11, 20, 21]. It was observed that by treating the polysaccharides obtained from a suspension of cultured sycamore cell walls with a α-1,4-*endo*-polygalacturonase, a polymer that had a core of alternating α-1,4-linked D-galacturonic acid and α-1,2-L-rhamnose units could be isolated. It also turned out [11], when studying the pectin polymer from different sources that they all had a striking similarity. The rhamnose units in the alternating core were frequently found as branch points, primarily on position 4, carrying galactan and arabinan side chains of varying structure. Rhamnose was occasionally branched on position 3 as well. The arabinogalactans attached to the rhamnose units are frequently found to be of the arabinogalactan type II (precipitates with the Yariv reagent), although AG-I occasionally also may be present. Both galactans with the main chain being 1,6-linked and 1,3-linked may be found for AG-II. This re-

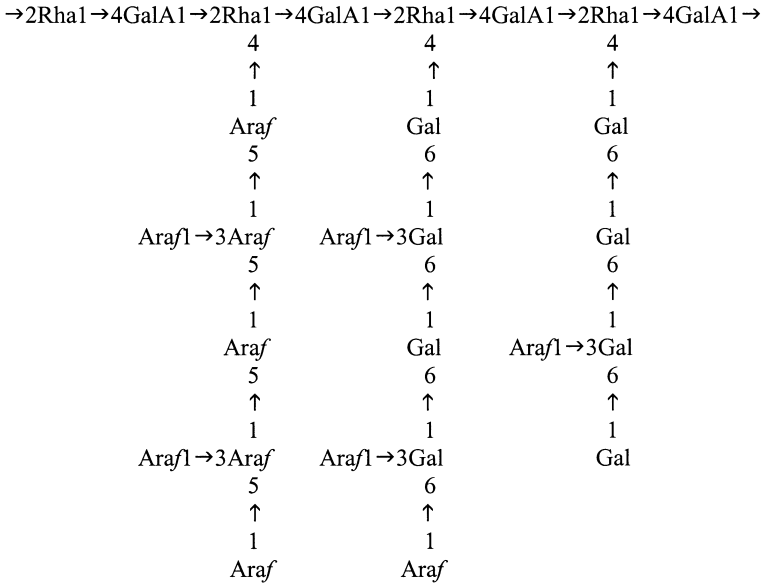

Fig. 2 Average structure of the "hairy" or "ramified" region of an apectic substance, with a rhamnogalacturonan I backbone substituted at position 4 of the rhamnose units with arabinan and arabinogalactan type II side chains

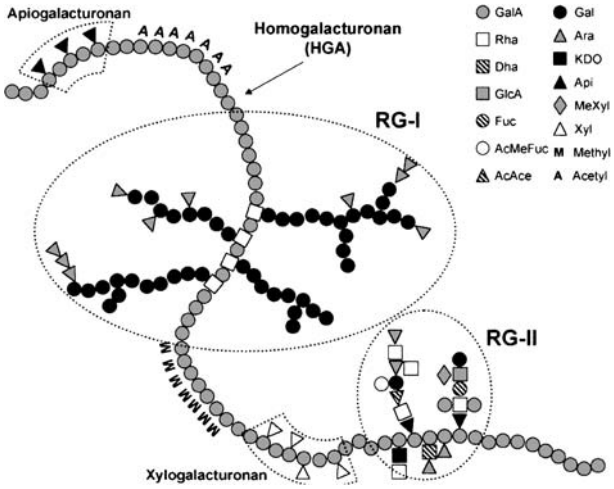

Fig. 3 A schematic presentation of the primary structure of pectins according to Perez et al. [23]

gion is now termed the "hairy" or the "ramified" region of the pectic polymer and an average image of RG-I is given in Fig. 2.

In addition to the hairy region, it was found that the pectic polymer contained a so called smooth region only composed of α-1,4-galacturonic acid residues. This smooth region can carry methyl ester groups and also be acetylated at positions 2 and/or 3 [22]. It has also been reported that the "homogalacturonans" can be substituted, often with single xylose residues. The position of these is schematically shown in the model of the pectic polymer as proposed by Perez et al. [23] in Fig. 3. This chapter will deal with bioactive pectins, and detailed structures of those containing the RG-I sequences will be discussed where relevant.

2.5 Rhamnogalacturonan II (RhaGalA-II, RG-II)

Rhamnogalacturonan II is a part of the pectin complex that cell wall polysaccharides are composed of, and comprise only a minor part of the total amount of pectins present. They have a so-called "homogalacturonan" backbone composed of 9–10 D-galacturonic acid units that are α-1,4-linked, and four different oligosaccharide chains are attached via positions 3 or 4 of the uronic acid backbone. As the backbone consists only of galacturonic acid, RG-II is not really an appropriate name for this polymer, but it has been kept as it was in use for a long time before the real structure of this polymer was discovered. The most characteristic part of RG-II is the presence of the rare sugars 2-*O*-methylfucose, 2-*O*-methylxylose, apiose, aceric

acid, 2-keto-3-deoxy-D-manno-octulosonic acid (KDO) and 3-deoxy-D-lyxo-2-heptulosaric acid (DHA) [11, 24]. The real structure, as it probably exists, as a three dimensional polymer was proposed by the group of Perez [25]. Figure 4 shows the primary structure of RG-II. This is the basis for the three dimensional orientation proposed that is composed of 2 units that appear to be linked via two apiosyl residues being cross-linked by a 1 : 2 borate diol ester. Apiose in the A chain (Fig. 4), is involved in the linkage although it is not in the centre of the molecule. This location leaves space for borate to be incorporated and as also shown by AFM in the same paper, creates a dimer structure that may be the basic structure for a possible extension to form a larger complex (Fig. 5). The data for the 3D-structure is basically deducted from NMR studies. RG-II is, as seen, a complex structure that appears to be present in the primary cell wall in most plants. Discussions on the biosynthesis and the high number of enzymes involved in producing this well conserved, complex oligomer are focussed on by Perez et al. [23]. The structure of RG-II that was isolated from red wine after the fermentation process, showed the presence of both the monomeric and dimeric substance. The reason for finding this high molecular weight oligosaccharide present after the fermentation, may be explained by the fact that RG-II is rich in the atypical monomers and oligomers mentioned above that are of such a nature that enzymes having those as substrates do not exist. Twenty-nine different plants

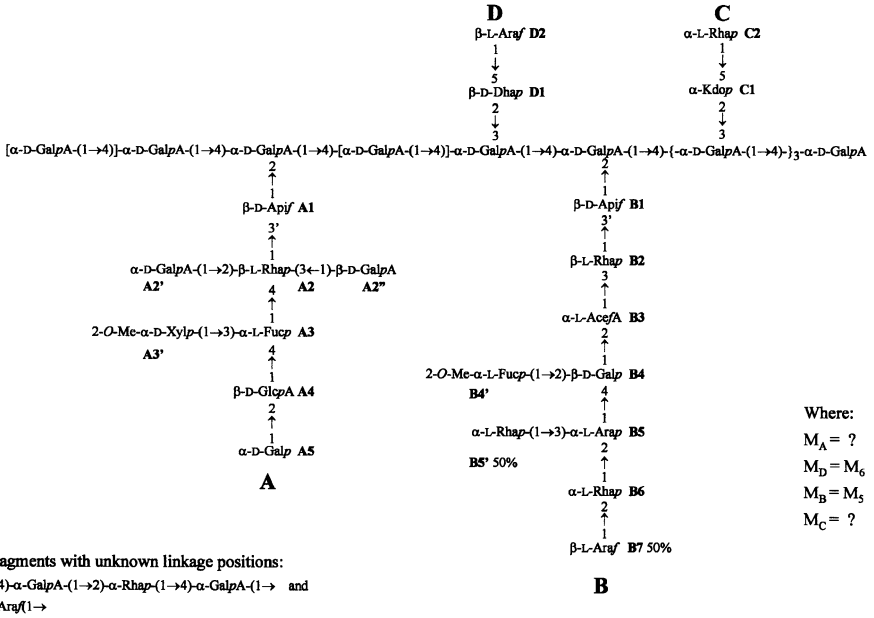

Fig. 4 The complete structure of rhamnogalacturonan II as described by Rodrigues-Carvajal et al. [25]

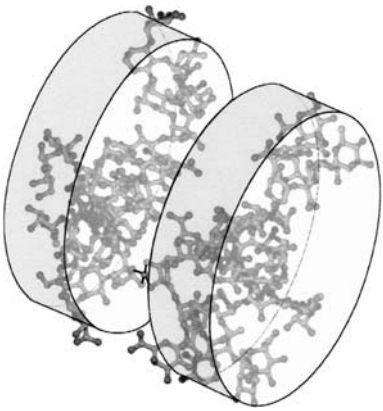

Fig. 5 Presentation of the 3D organisation of the RG-II dimer in which two apioses are cross-linked by a 1 : 2 borate-diol ester, as proposed by Perez et al. [23]

have been reported in the literature up to 2003 to contain RG-II with more or less the same structure.

Various pectin containing plants that are used in traditional medicine have been shown to contain RG-II. The pectic substance Bupleuran 2IIc from *Bupleurum falcatum*, having an effect on the gastric ulcer, and studied in great detail by the group of Yamada contains a minor region of RG-II. *Atractylodes lancea* pectin, ALR-b, and leaf pectin from *Panax ginseng*, both contain RG-II in the bioactive fraction. So does *Angelica acutiloba* and *Glycyrrhiza uralensis* [26–28]. The bioactivity of these polymers will be discussed below, as will their structure activity relations.

3
Bioactive Pectic Polysaccharides Isolated from Plants

As mentioned in the introduction, various reviews over the last ten years show that many plants contain bioactive polysaccharides. Most of the plants studied were chosen due to their traditional use for different kinds of illnesses where the immune system could be involved. The following section will describe the pectic type polymers from the plants most studied for their structure, and activities related to the structure where possible.

3.1
Acanthus ebracteatus Vahl, Acanthaceae

Acanthus ebracteatus is a plant traditionally used for various ailments, amongst those skin diseases in Thai traditional medicine. The stem of the plant was shown to contain neutral and acidic polysaccharides with effect in

the complement system that was quite high compared to the normal standard used. The neutral polymer is composed of galactose, 3-*O*-methylgalactose and arabinose in the ratio 3 : 4 : 1. Both galactose and 3-*O*-methylgalactose are mainly β-1,4-linked, arabinose α-1,5, in addition they are also terminal groups and branch points, for the galactoses mainly on position 6. Fractionation by gelfiltration of the slightly more active acidic fraction gave six different subfractions, of which the highest Mw fraction ($\sim$ 1500 kDa), A1002a, had the lowest concentration of galacturonic acid and the highest of 3-*O*-methylgalactose of the subfractions obtained. It also had the highest activity of all, significantly higher than those with an Mw in the region 30–60 kDa. These latter had comparable activity with the standard (PMII from *Plantago major*) used, and had also similar Mw, monosaccharide composition and structural features as PMII. Both the high amount of the unusual sugar 3-*O*-methylgalactose, and the high molecular weight of the most active polymer from *A. ebracteatus* may be important factors for the activity observed [29, 30].

3.2
Angelica acutiloba Kitagawa, Umbelliferae

Immunostimulating polysaccharides were already in 1982 observed in the water extract of roots from *Angelica acutiloba* [31]. This paper was the basis for further studies on the polysaccharides from this plant. One of the first arabinogalactans for which an activity on the complement system was shown, was in fact an arabinogalactan from a hot water extract of the roots of this plant [32]. The polymer was called AGII and was, although only a minor component of the total amount of polysaccharides present in the root, the most potent one. The polymer was shown to be an arabino β-galactan, the backbone most probably composed of an α-1,6-linked galactan with α-1,5-arabinofuranosyl residues linked through C-3 of the galactan backbone. This polymer activates the complement system both via the alternative and classical pathways and contains structures similar to the general AG-II polymers. The polymer was subjected to degradation both by mild acid hydrolysis and enzymatic digestions. Linkage analyses of the obtained oligomers gave the following basic structures: a 1,6-linked galactan with unbranched short side chains of Ara*f* on position 3; 1,4-linked gal was also observed, as well as highly branched Ara*f*-oligomers with linkages both on positions 3 and 5. Base catalysed degradation gave rise to rhamnogalacturonan I sections having substitutions on position 4 of the rhamnose units [33]. Other pectins with effect on the complement system isolated from the roots of *A. acutiloba* were composed of over 90% of a galacturonan region with a small amount of the ramified region [34]. The ramified region, isolated after degradation of the polysaccharide with pectinase and pectinesterase, contained the rhamnogalacturonan core possessing side chains rich in neutral carbohy-

drate chains, which were directly attached to position 4 of rhamnose [35]. The ramified region from each pectin had a more potent complement-activating effect than the corresponding original pectins, and the oligogalacturonides had weak or negligible activities. These facts suggest that the complement-activating potency of these pectins is expressed mainly by their ramified regions [34]. The total structure of this polymer has been proposed by Kiyohara and Yamada [36]. The relationship between the structure and the effect on the complement system of this polymer is discussed in great detail [37]. Removal of the external Ara*f* units of the molecule resulted in higher activity than that of the "mother-molecule", the arabinogalactan side chains, AG-I type, showed the most potent activity of the side chains prepared. Degradation of the rhamnogalacturonan core decreased the activity slightly, the two acidic arabinogalactan units comprised of highly active arabinogalactan and galactan side chains. It was concluded that the 1,3,6-β-galactan moiety of the arabinogalactan side chains contributed the most to the effect after the degradation. When the 1,6 D-galactosyl side chains were removed, the activity was not altered, intact 1,6 galactan chains had negligible activity, and it was thus concluded that the 1,3-β-D galactan backbone was essential for the activity. The "mother-molecule" AGIIb-1 mainly expresses the effect on the complement system via the classical pathway, while the one obtained after enzymatic removal of the external arabinose moieties expressed its activity via both classical and alternative pathways. The suggestions from these findings were that the 1,3,6-β-D-galactan moiety is involved in the expression of activity via both pathways, while the Ara*f* side chains may inhibit the expression of the activity through the alternative pathway. In other studies a reduction of the activity was seen after removal of the galactose units by the *exo*-β-1,3-galactanase, but the digested product was still active. These results also showed that 1,6-linked galactose side chains are important for the activity, and that the attachment of those to a 1,3 galactan backbone is necessary for the optimum activity [38].

As most of the complement activating polysaccharides present in *Angelica acutiloba* contain pectin with "ramified" regions also being important for the bioactivity of the polymers, Kiyohara et al. [39] studied in detail the relationship between structure and activity of these regions. They found that digestion of the polymer called AR-2IIa with *endo*-α-D-1,4-polygalacturonase gave rise to a ramified region called PG-1a, being a rhamnogalacturonan with neutral side chains, and oligogalacturonides. The resistant product, E-PG-1a, obtained after degradation with *exo*-α-L-arabinofuranosidase and *exo*-β-D-galactosidase had the same effect on the complement system as the ramified region itself. This core contained both long and short galactosyl chains consisting of a non-reducing terminal, 1,6-linked and 1,3,6-linked units for the long chains and the short ones contained basically 1,6-linked units. Degradation of the GalA moieties in PG-1a decreased markedly the effect on the

complement system, while the long and short galactosyl chains expressed ~ 50 and ~ 20%, respectively, of the activity of E-PG-1a.

It is interesting to note that another root pectic polysaccharide from the same plant also expresses antitumour activity against an ascitic form of Sarcoma-180, IMC carcinoma, a Meth A fibrosarcoma, as well as the solid form of a MM-46 tumour [40]. Structural studies on this polymer showed that it consists of a rhamnogalacturonan moiety with branches on C4 of rhamnose, and also contained a highly branched 3,5 arabinan and a 1,4-linked galactan, indicating an arabinogalactan type I (AG-I) structure. It also contains 1,3, 1,6 and 1,3,6-linked galactose units, which were also present in the pectic polymer with effect of the complement system. It was interesting, though, that the antitumour polysaccharide did not have significant effect on the complement system, indicating that the neutral 1,4-linked galactose chains may be of importance for the anti-tumour activity. Early studies on pectins from Japanese medicinal herbs indicated that *A. acutiloba* polysaccharides contained RG-II, but activities related to this structural element have not been pursued further [41].

3.3
Atractylodes lancea DC Asteraceae

The rhizomes of the plant *Atractylodes lancea* were shown to contain three polysaccharides that contributed to the expression of the immunomodulating activity that was found from a preparation of traditional Japanese Kampo medicine [42]. The test system for the immunomodulating activity was based on the ability of the polymers studied to express a stimulating effect on the cytokine production of the Peyer's patch cells. One of the polysaccharides was characterised as an arabino-3,6-galactan (AG II). After removal of the outer Ara*f* units and treatment of the remaining polymer with an *endo*-β-D-1,6-galactanase, the activity was remarkably decreased. Structural analyses showed that the removed side chains were mainly composed of β-D-1,6-galactopyranosyl oligosaccharides with a DP ranging from 1 to 8 units. Degradation of the β-D-1,3 galactan backbone also reduced significantly the activity, indicating that some of the side chains attached to the backbone were also responsible for the activity. The basic structure of the polymer is a β-D-1,3 galactan backbone with β-D-1,6-galactopyranosyl side chains attached, and these are again decorated with arabinofuranoside residues, the major part being terminal units [43, 44].

Two pectic polysaccharides (ALR-a and ALR-b) were also responsible for intestinal immune system modulating activity [45]. The pectins were degraded with an *endo*-α-D-1,4-polygalacturonase, that on gelfiltration gave three fractions. The highest Mw fraction of ALR-b was shown to consist of the hairy region, RG-I, and the LMW fraction of oligogalacturonans, primarily. None of these fractions showed any intestinal immune system modulating ac-

tivity, while the intermediate molecular weight fraction, called PG-2, showed a potent effect in the same system. Chemical analysis of this showed that it contained several of the sugars normally found in rhamnogalacturonan II (RG-II), but did show differences to this one on the linkage type, and also by lacking some of the sugars normally found in RG-II. Gelfiltration and anion exchange chromatography of the enzyme degraded ALR-a resulted in a fraction called ALR-a-Bb with potent intestinal immune system modulating activity through the Peyer's patch cells. This fraction resembled RG-II based on the monosaccharide composition in the same way as PG-2 did. A review of these bioactive polymers has also been written [46].

3.4
Bupleurum falcatum L. Umbelliferae

The roots of the Sino-Japanese medicinal herb have a long tradition for use in the treatment of chronic hepatitis, nephrotic syndrome and various auto-immuno diseases. Yamada's group have extensively studied the polysaccharide responsible for the anti-ulcer and mitogenic effect shown for the active principle in the roots. The polysaccharide fraction responsible was denominated Bupleuran 2IIc, and was shown to be a pectic type polymer. It consists of approx. 70% α-1,4-linked galacturonic acid, of which 30% are methyl-esterified. Parts of the galacturonic residues present are also branch-points. Bupleuran 2IIc also contains ramified or hairy regions consisting of a rhamnogalacturonan core having neutral side chains of mainly galactose and arabinose units, attached to the 2-linked rhamnose units in the main core or to the 4-linked galacturonic acid residues. This region has structural elements typical of the rhamnogalacturonan type I (RG-I) pectins. The Bupleuran 2IIc also contains a minor region with similarities to the pectic rhamnogalacturonan II (RG-II) that contain the rare sugars KDO, DHA, Apiose and Aceric acid [46, 47] (and refs. cited therein). Bupleuran 2IIc was shown to have potent complement activating and antiulcer activities. The mechanism behind the mucosal protection was suggested to be due to its anti-secretory activity on acid and pepsin, its increased protective coating and its radical scavenging effect, but was not involved in the action of endogenous prostaglandins and mucus synthesis [46] (and refs. cited therein). As the traditional medicines are mainly taken orally, they also studied the possible uptake and tissue distribution of the Bupleuran 2IIc by means of a polyclonal antibody raised in rabbits by injection of the hairy region obtained after *endo*-polygalacturonase treatment of the native polymer [48]. When Bupleuran 2IIc was intravenously injected into mice, the polysaccharide disappeared from circulation within 24h, and was mainly detected in the liver by an ELIZA method based on the antibody raised. When a crude mixture containing mainly Bupleuran 2IIc was administered orally to the mice, the polysaccharide was detected in the liver and in the Peyer's patches. It

was found that the outer part of the hairy region consisting of two different oligosaccharide chains having GlcA or 4-O-MeGlcA at the non-reducing end bound to 6-linked galactose units were the antigenic epitopes of the anti-ulcer polysaccharide Bupleuran 2IIc, Fig. 6 [49]. This verifies that the polymer can have an effect when administered orally.

The other effect that was substantially studied was the mitogenic effect of the polymer. When mice were fed the polysaccharide for 7 days, proliferative responses of the spleen cells were enhanced. In vitro studies showed that Bupleuran 2IIc proliferates B-cells in the absence of macrophages, and the activated B-cells are induced into anti-body forming cells in the presence of IL-6. Bupleuran 2IIc in the presence of the antibody raised against the hairy region gave a reduced mitogenic activity. The mitogenic activity was

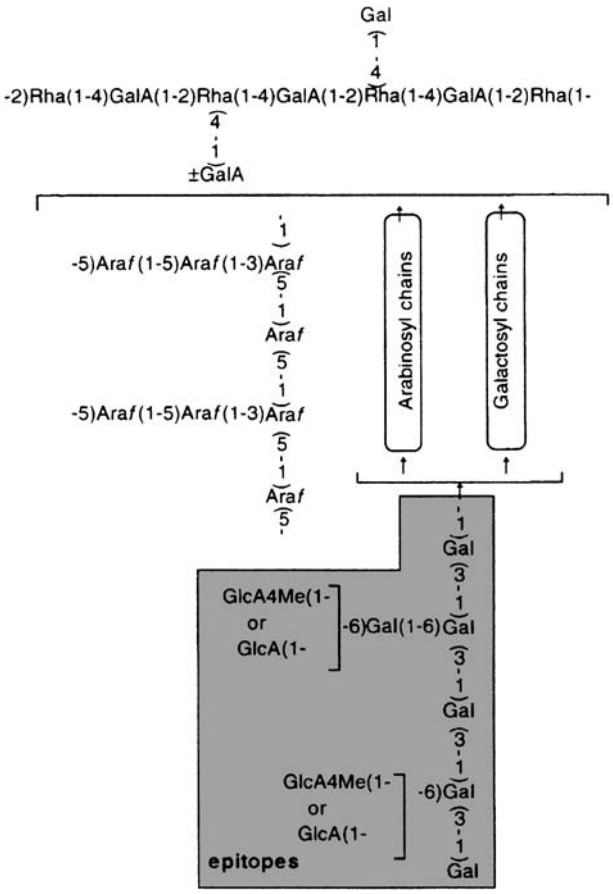

Fig. 6 Proposed structure of the antigenic epitopes in the "ramified" region of bupleuran 2IIc for anti-bupleuran 2IIc/PG-1-IgG as proposed by Sakurai et al. [49]

also reduced in the presence of β-D-Glc*p*A-1,6-β-D-Gal*p*-β-D-1,6-β-D-Gal*p* or the dimer with one gal*p* unit, showing that the epitope on Bupleuran 2IIc that was recognised as the antibody binding part of the molecule also acts as the active site of the polysaccharide for the mitogenic activity. Bupleuran 2IIc also enhances the IgM secretion from highly purified murine normal B-cells. The hairy or ramified region of the polysaccharide showed potent IL-6 secretion-enhancing activity, indicating that the same active site as above may partly contribute to the enhancement of IgM secretion as an autocrine and/or paracrine mechanism [50].

3.5
Cistanche deserticola Y.C.Ma. Scrophulariaceae

This organism is a holoparasite that grows on the roots of the hardwood *Haloxylon ammodendron* that is widely distributed in the Gobi desert, Mongolia, and has a wide use as a traditional medicinal remedy. The drug was found to contain various polysaccharides of pectic nature with mitogenic and comitogenic effect using Zymosan as a positive control. Methylation and NMR spectroscopic studies show that the pectic type polymers in *C. deserticola* most probably are of the rhamnogalacturonan type I, having side chains both of arabinogalactan type I and type II. Methyl-esters and acetyl-groups are identified by carbon-NMR spectroscopy [51]. Further studies lead to the isolation, after enzymatic degradation for removal of starch and protein, different precipitation methods, ion-exchange chromatography and gelfiltration, of a pectic arabinogalactan called Cistan A. This polymer can be characterised as an immunomodulating pectic substance based on the dose-dependant mitogenic and comitogenic effect found that is higher than the standard used. The methylation analysis of Cistan A shows that this molecule does not contain the common features of the Rhamnogalacturonan I type polymers as the rhamnose basically is 1,4-linked, not 1,2 as is common in the RG-I type. The arabinose and galactose linkages confirm that both AG-I and AG-II polymers are attached to the main core of Cistan A. Other bioactive fractions were also obtained, their activity was lower than that of the Cistan A. Their monomeric composition were fairly similar apart from one having a higher content of xylose than the other [52].

3.6
Cuscuta chinensis Lam. Convolvulaceae

Polysaccharides isolated from the seeds of *C. chinensis* have effects both as immunostimulants and as antioxidants. The polysaccharide CS-A-3-β has a backbone of α-D-1,4-linked Gal*p*A and β-L-1,2-Rha*p* units with branches at C-4 of the Rha*p* residues and at C-3 of Gal*p*A residues that are composed of an arabinogalactan and glucobiose. The Ara*f* units are terminal and 1,5-

linked, while the Gal units are terminal, 1,6- and 1,3,6-linked, typical for the AG-II type polymer chains as part of the rhamnogalacturonan type I (RG-I) polysaccharide. The glucobiose is α-1,4-linked and may be linked to the GalA units. The effect of the polysaccharide fractions on hydrogen peroxide induced cell lesion of rat pheochromocytoma line PC 12 was tested. Pretreatment of the cells prior to exposure with hydrogen peroxide gave an enhanced cell survival when the pure CS-A-3b was used, showing that this polysaccharide has a protective effect against hydrogen peroxide induced cell toxicity on PC 12. The glucobiose on the GalA units is not common for pectins and is a characteristic feature for this polymer that may be partly responsible for the effect seen. This polysaccharide was probably the first reported to protect resting nerve cells from free radical-induced injury. Further structural studies will reveal the part of the pectin that is responsible for the bioactivity shown [53].

3.7
Diospyros kaki L. Ebenaceae

D. kaki leaves contain a polysaccharide that has a backbone of alternating α-1,4 Gal*p*A units and α-1,2 Rha*p* units. Most of the Rha units are branch-points with branches on C4, consisting of β-1,4-linked xylose units, and others consisting of β-1,3 and β-1,6-linked galactose units. These side chains are also substituted with Ara*f* on O-2 of the xylose units and O-3 on the β-1,6 Gal units. The structure proposed is not common amongst the rhamnogalacturonans as the side chain on the xylose units appear to be rare. The polysaccharide stimulates the LPS-induced B-lymphocyte proliferation, but not ConA-induced T-lymphocyte proliferation. It is proposed that the labile Ara*f* units not are important for the expression of the enhancement of the immunological activity, the presence of GalA in the backbone appears to have an important, but not a crucial effect on the expression of the activity. Further studies will have to be performed on this polymer in order to verify this new type of pectic polymer [54].

3.8
Entada africana Guill. et Perr. Mimosaceae

The polysaccharides from *Entada africana* were isolated by water extraction and further separated by anion exchange chromatography. The fraction called EA 100 Acidic 1, had an effect in the complement system equivalent to the standard polymer used. This polymer was shown to basically be an arabinogalactan protein with a relatively high degree of xylose. The ratio ara : gal : xyl was 3 : 2,5 : 2 and the protein part was rich in hydroxyprolin. Linkage analyses as well as precipitation with the Yariv reagent showed that this polymer belongs to the AG-II type. The xylose present was 1,4-linked. Removal of Ara*f*

units by weak acid hydrolysis converted most of the 1,4-xylose units into terminal ends, indicating that araf was linked on position 4 of single xylose units. It was also obvious that the Ara*f* units were linked through both positions 3 and 4 of galactose, and in addition to the presence of 1,3,6-linked galactose, 1,4-linkages were also present, indicating that part of the fraction contains AG-I structures. Removal of the Ara*f* units by acid hydrolysis decreased the activity in the complement system. This may be caused either by the denaturing of the protein part or the fact that Ara*f* units influence the activity. The polysaccharide fractions extracted from *E. africana* also contained polymers of the RG-I type, but the activity in the complement system for these was minor compared to that of EA 100 Acidic 1 [55].

3.9
Glinus oppositifolius (L.) Aug. DC. Aizoaceae

The West-African plant *Glinus oppositifolius* has been used, amongst other ailments, in the treatment of wounds. The plant was shown to contain polysaccharide fractions that gave a high effect in the complement system. Two pectic type polysaccharides, GOA1 and GOA2, was isolated by different chromatographic methods. The polysaccharide GOA1 contains terminal, 1,3- and 1,5-linked Ara*f* and 1,4-, 1,3- and 1,3,6-linked Gal*p*, suggesting the presence of both AG-I and AG-II type arabinogalactans. The GOA2 is supposed to be an RG-I type pectic polymer with side chains of AG-II structures attached to position 4 of the rhamnose units in the main core. In addition to the potent complement fixing activity, both polymers were shown to have chemotactic properties towards macrophages, T-cells and NK-cells, showing that both polymers have immunomodulating abilities [56].

3.10
Glycyrrhiza uralensis Fisch ex DC. Fabaceae

The first paper on the bioactive polysaccharides from *Glycyrrhiza uralensis* roots was published in 1996 by Kiyohara et al. [57]. They isolated a pectic type polymer with anti-complementary and mitogenic activity that was an acidic pectin, possibly containing rhamnogalacturonan type I as part of the total structure. Degradation of the uronic acid part of the molecule decreased both types of bioactivities. The neutral oligosaccharide chains were shown to retain some of the activities of the native polymer, but it was suggested that they should be attached to the acidic core to retain maximum activity.

It was also shown that the pectic type polymers from *G. uralensis* contain a minor part of the RG-II type structure [26].

In a more recent study it was shown that the roots from *G. uralensis*, after isolation, fractionation and purification, contain two bioactive polysaccharides of the pectin family termed GU-3IIa-2 and 3IIb-1.

They are both immunologically active, but they did react differently in the different test systems used. GU-3IIa-2 was a potent bone marrow cell proliferating activity enhancer, while GU-3IIb-1 was a potent NK cell-mediated tumour cytotoxicity enhancer. 3IIa-2 is mainly composed of arabinose (35%), galactose (25%) and galacturonic acid (15%). The polymer contains arabinogalactan type II as it forms a precipitate with the Yariv reagent, and is also composed of terminal, 1,5 and 1,3,5-linked arabinose units, and a high degree of 1,3,6-linked galactose units in addition to terminal, 1,3-, 1,4-, and 1,6-linked units. The galacturonic acid is mainly 1,4-linked, with some terminal and 1,2,4-linked units.

3IIb-1 is a more complex pectic type polymer. It contains the most common types of linkages for acidic pectins and it does not precipitate with the Yariv reagent and methylation studies shows that the galactan moieties are rich in 1,4 linkages, typical for the arabinogalactan type I polymer. This bioactive polymer is most probably a typical rhamnogalacturonan type I containing AG-I structures. Detailed structure activity relations of both of these polymers await further studies [58].

3.11
Larix occidentalis Nutt, Pinaceae

Larch arabinogalactan has a structure (Fig. 7) that can be classified as an arabinogalactan type II, but it differs from other AG-II's so far found in the plant kingdom as the larch arabinogalactan will not precipitate with the Yariv-reagent, and it does not have an effect on the complement system, as reported by Yamada et al. [4]. The larch arabinogalactan has been shown to have various other interesting biological activities making the polymer an excellent source as a dietary fiber, and as such has been approved by the FDA. It increases the production of short fatty acids, and decreases the generation and absorption of ammonia. It also appears to have a significant effect on enhancing beneficial gut flora, especially the *Bifidobacteria* and *Lactobacillus*. Experimentally it has been shown that the larch arabinogalactan can stimulate natural killer cell cytotoxicity and other functional aspects of the immune system, and also inhibit the metastasis of tumour cells to the liver [59].

A monograph on the larch arabinogalactan has been published, containing pharmacokinetics, clinical indications, the lack of side-effects and dosage [60]. In a report from 2003, the effect over time of in-vivo administration of the larch arabinogalactan on the immune and hemopoietic cell

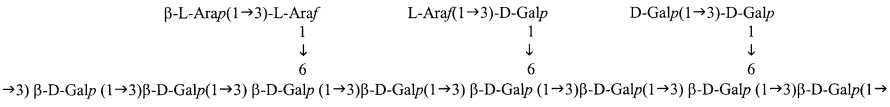

Fig. 7 Structure of the Arabinogalactan type II polymer from Larch

lineages in murine spleen and bone marrow is discussed. Results show that 7–14 days after administration to mice, lymphoid cells in the bone marrow were significantly decreased relative to control, but remained unchanged at both time intervals in the spleen. NK cells were also, after 7 days exposure, decreased significantly in the bone marrow, but not in the spleen. After 14 days the NK cells in the bone marrow returned to a normal level, but were increased in the spleen.

A vast cascade of cytokines appear to be induced by the presence of this polysaccharide, and immunopoiesis- and hemopoiesis-inhibition are probably the most prevalent during the first two weeks of daily exposure [61]. Studies relating structure to the biological activity have not been performed.

3.12
Lycium barbarum L. Solanaceae

The fruit of the medicinal plant *Lycium barbarum* contains an arabinogalactan protein, for which the carbohydrate part was classified as an arabinogalactan type I polymer. The structural features of the carbohydrate part are somewhat unusual as it is proposed, from the data obtained, to consist of a backbone of β-1,4-linked galactose units, and all units are branched at position 3 with chains of different compositions, see Fig. 8. The native polymer was shown to promote splenocyte proliferation directly in normal mice, and the carbohydrate part of the polymer had a stronger effect than the glycoconjugate, showing that the immuno-modulating effect of the glycoconjugate resides in the arabinogalactan moiety. Experiments also showed that the most likely target cells were the B-lymphocytes that appear to carry receptor binding sites acting with the polymer [62].

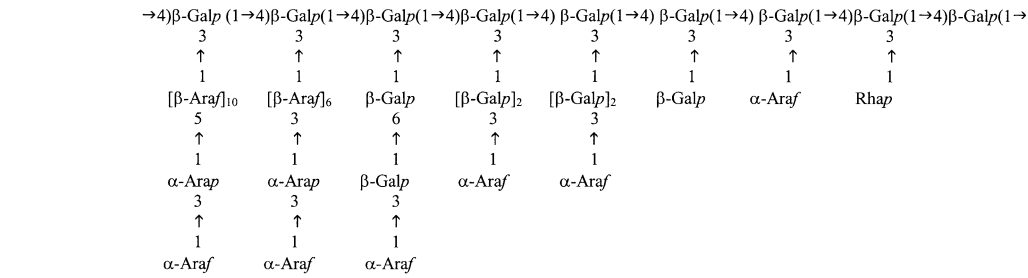

Fig. 8 Arabinogalactan from *Lycium barbarum* as proposed by Peng et al. [63]

3.13
Melocactus depressus Hook, Cactaceae

The pulp of this cactus contains an arabinogalactan type I polymer with the ability to stimulate phagosytosis. The galactose units are 1,4-linked with

branches on position 3 on some of the galactose units and on position 6 on others. The side chains are proposed to be trisaccharides consisting of an Ara*f* unit that has terminal Ara*f* substituted on positions 2 and 5 in some cases and 3 and 5 in others [63].

3.14
Panax ginseng C.A.Meyer, Araliaceae

Ginsenan S-IIA, a polysaccharide fraction from the roots of *P. ginseng* is a potent inducer of IL-8 production by human monocytes and THP-1 cells, and this induction is accompanied by increased IL-8 mRNA expression. The polysaccharide appears from the structural feature to be a mixture of arabinogalactan type I and type II, based on the presence of 1,3-, 1,6-, 1,3,6-, 1,4-, and 1,4,6-galactose units as well as terminal arabinose and 1,5-, 1,3,5-, and 1,2,5-linked units. It also contains 1,4,6-linked glucose units that together with the 1,2,5-linked arabinose units are different from the units found in other ginseng polysaccharides and may thus be of importance for the activity [64].

Already in 1988 and 1991, Gao et al. [65, 66] detected four different polysaccharides present in the leaves of *Panax ginseng* that had an effect on the complement system, but only two of them, the neutral, GL-NIa, and one of the acidic ones, GL-AIa, had potent activities at low concentrations. GL-NIa was found to be mainly an arabinigalactan type II polymer. GL-AIa was a polysaccharide with a rhamnogalacturonan core with neutral side chains of the AG-II type, confirmed by a strong reaction with the Yariv reagent and the methylation results. It was shown that the crude polysaccharide fraction contained KDO and DHA, suggesting the presence of Rhamnogalacturonan II in

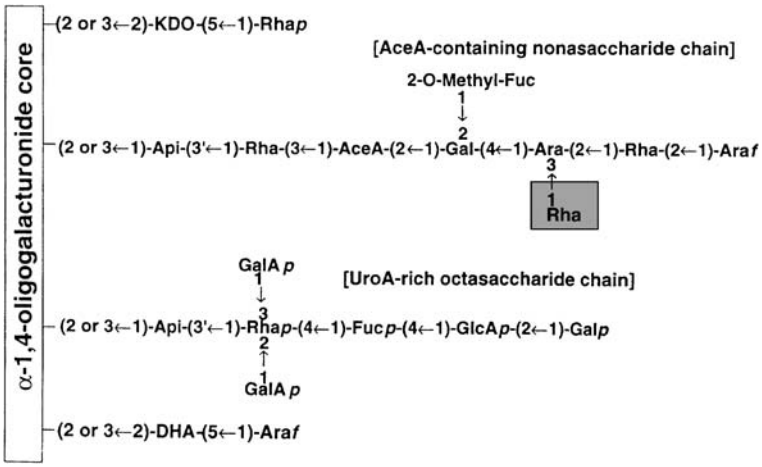

Fig. 9 Rhamnogalacturonan II from the leaves of *Panax ginseng* as proposed by the group of Yamada [3]

the fraction. Three different RG-II polymers were isolated without the digestion of the native polymer with *endo*-polygalacturonase [67, 68]. One of these, denominated GL-4IIb-2, was a macrophage Fc receptor expression enhancing polysaccharide, and also had the most potent IL-6 production enhancing activity of macrophages of the polymers isolated [67, 69]. The structure of this RG-II (Fig. 9) was shown to be slightly different in the structure than the RG-II shown in Fig. 4. The active polymer was present as a dimer with the borate linking the apiose of the two units together. Dissociation led to diminished activity, while redimerisation recovered the activity [68]. As other RG-II dimers were tested for the same activity giving no effect, it was suggested that the specific structure of the GL-4IIb-2 dimer was necessary for the activity [68]. Yamada's group has also found that this polymer shows a potent secretion enhancing activity of the nerve growth factor (NGF) which is known to play a role as a neurotrophic factor for the survival of neuronal cells and preventing aging and dementia, indicating that the polysaccharides of ginseng may have several interesting biological activities [3].

3.15
Piper nigrum L. Piperaceae

Black pepper contains several polysaccharides of which one shows a strong effect as an immune enhancer based on the fact that the polymer is an anticomplementary polysaccharide. The polysaccharide has an Mw of approx. 40 kD. It is composed basically of rhamnose, arabinose, galactose and galacturonic acid, and shows a high binding capacity for the Yariv reagent. This indicates that the side chain of the polymer is of the arabinogalactan type II, which is a common structure for several polysaccharides with an effect on the complement system [70].

3.16
Plantago major L. Plantaginaceae

The leaves of *Plantago major* have a long tradition in most areas where the plant grows as a woundhealing agent [71–73]. Two different polymers of the pectic type with high activity in the complement system are present in the leaves of *P. major*. PMIa was shown to be an arabinogalactan type II polysaccharide, it gave a positive Yariv reaction, and is composed of a galactan 1,3 backbone, heavily branched with 1,6-linked side chains linked through O-6 of the galactan core. To these side chains, terminally and 1,5-linked arabinofuranoside-residues are linked through position O-3 of the 1,6-linked galactan side chains. The polymer contains a protein part that is rich in hydroxyproline, serine and alanine, being typical of AG-II polymers [71].

The other polymer denominated PMII [18, 72, 73], is a pectic type polymer with mainly a galacturonic acid backbone. The structure of PMII was

found to be similar to the general type described by Voragen et al. [22] with a smooth region, consisting of α-D-galacturonosyl units 1,4-linked and with hairy regions. Two different types of hairy regions were isolated after degradation of the polymer with pectinesterase and pectinase. The main core in both regions were alternate 1,4-linked galacturonic acid units and 1,2-linked rhamnose units. The main difference structurally between the two types of hairy regions were the molecular weight, the GalA/Rha ratio, being higher for the compound with the lowest molecular weight and a lower degree of substitution of the rhamnose units for the same fraction. The one with the largest Mw was called PVa, the smallest PVb. The first was more active in the complement system than the latter. The structure of PVa resembled the RG-1 structure shown above.

Arabinofuranosides were removed from PVa, which resulted in an increase in the activity; this was in contrast to the findings of Kiyohara et al. [35] who found no change in activity after removal of similar units from *A. acutiloba* pectin. For this polymer it was suggested that the minimum requirement for complement activation via the classical pathway was β-1,6-linked galactan attached to the rhamnogalacturonan backbone, which also appears to be an important part of the backbone for PVa.

More detailed studies on the interaction between human complement and PMII [74] showed that PMII appears to be an activator both on the classical and the alternative pathway. The complement activation was performed using serum from ten different individuals as the complement source. The surprising result was that the activation differed considerably depending on the complement source, a 200-fold difference in ICH_{50} value was observed. The levels of antibodies against PMII detected in the different sera did not correlate with the levels seen for ICH_{50} activity of PMII. PMII appears to be as potent an activator of complement as aggregated human immunoglobulin, (IgG), and these results might be related to the reported wound-healing effect of the leaves of *Plantago major*. An in vivo study was also performed, and the results demonstrated that PMII protects against pneumococcal infection in mice when administered systemically prechallenge, and also that the protective effect was owing to stimulation of the innate and not the adaptive immune system [75, 76].

3.17 *Salvia officinalis* L. Lamiaceae

The aerial part of sage was successively extracted with water, potassium oxalate, DMSO and KOH, and gave rise to different fractions with bioactivity. Partial purification of the water extract gave rise to a polydisperse fraction called A, that based on the monosaccharide composition, IR, and NMR, was thought to be composed of arabinogalactans associated with the highly ramified rhamnogalacturonans core. Fraction B, extracted with oxalate, was

characterised (based on the same information as for A) as a typical pectin material with arabinan side chains. Both of these polysaccharide fractions are thought to be partly responsible for the immunomodulatory effect of the sage extract. The effect was shown by the mitogenic and comitogenic activity the polymers exhibited [77].

3.18
Tinospora cordifolia Miers, Menispermaceae

The stems of the tree were found to contain polysaccharides consisting of arabinose, galactose and galacturonic acid and only minor amounts of rhamnose. Structural studies indicate that the polymeric material consists of 1,4-linked galacturonic acid residues, terminal, 1,4-, 1,6- and 1,3,6 galactose units and terminal and 1,5-linked arabinofuranose residues. Further studies must be performed on this in order to determine what type of pectin it can be classified as. The linkage data indicate that both AG-I and AG-II are present. This polymer was shown to activate polyclonal B-cells [78].

The antioxidant properties of the polysaccharides against iron-mediated lipid damage and γ-ray induced protein damage was studied. The polysaccharide was shown to be a good protector against the iron-mediated lipid peroxidation of rat brain homogenate revealed by the thiobarbituric acid reactive substances and the lipid hydroperoxide assays. The polysaccharide provides a significant protection to proteins against γ-ray induced damage. The protective effect was explained by the high reactivity towards DPPH, superoxide radicals and the most damaging of the radicals, the hydroxyl radical [79].

3.19
Trichilia emetica subsp. suberosa JJ de Wilde, Meliaceae

The leaves of the tree *T. emetica* have a traditional use as a wound healer, and is especially useful against old wounds, both infested and cancerous ones. The polysaccharide content of the leaves was shown to have an effect on the complement system [80] and was studied further. Both extractions with water (50 and 100 °C) gave an active fraction. Anion exchange chromatography of the material extracted at 100 °C leads to four different fractions with varying activities. Structurally, all acidic polymers have similar features with 1,4-linked galacturonic acid as the main constituent and rhamnose both 1,2 and 1,2,4-linked, indicate that the polymer is of the RG-I type with side chains on C-4 of rhamnose. The side chains appear to be of the AG-II type having all the relevant structural features of this as described above. Removal of the Ara*f* units by weak acid hydrolysis reduced the effect on the complement system, indicating that these units may play a role in the activity. It appears that the arabinose units primarily are linked through position 3 on 1,6-linked

galactose units, as the 1,3,6-linked units were substantially reduced for all polymers with a concomitant increase of the 1,6-linked units. No alteration of the amount of 1,3-linked galactose was seen indicating a backbone of 1,3-linked units with side chains of 1,6-linked galactoses that are decorated with ara*f* units [80].

3.20
Vernonia kotschyana Sch. Bip. Ex Walp.
Baccharoides adoensis var. *kotschyana* (Sch. Bip. Ex Walp.), Asteraceae

The roots of this plant are extensively used in Mali for the treatment of gastrointestinal disorders and wound healing and are part of a registered improved traditional medicine (ITM) called Gastrocedal. Water extracts of the roots gave rise to acidic polysaccharide fractions that showed a dose dependant activity in the complement system. The monosaccharide composition revealed those typical for pectic substances in addition to a high content of fructose, which was shown to be an inulin that was inactive in the test systems used in this paper. The Yariv reagent revealed that all fractions contain AG-II type polymers. The acidic fractions denominated Vk50A2 and Vk100A2 showed mitogenic activity as they proliferated spleen cells in a dose-dependant manner. When the cell population of the spleen cells responding to the stimulation by the two polysaccharides was investigated by flow cytometry analysis, the population of B-cell positive cells increased during the experimental period. An increase in apoptotic cells was not observed suggesting that the increase of B-cells was not due to a decrease of T-cells. Further, it was found that the polysaccharide fractions could be characterised as B-cell mitogens, and that the T-cells and macrophages were not involved in the stimulation. The induction of the lysosomal enzyme activity in macrophages was dose dependant, but the activity somewhat lower than the positive control used [81]. Further studies of the Vk100A2 fraction lead to the isolation of two fractions by size exclusion chromatography, Vk100A2a and Vk100A2b. The latter, containing mainly galacturonic acid, $\sim$ 85%, and smaller amounts of the neutral sugars arabinose, galactose and rhamnose, showed a complement fixing ability, but no activity on the proliferation of B- and T-cells. Vk100A2a showed a dose-dependant complement fixing ability and a T-cell independent induction of B-cell proliferation. Both polymers induced chemotaxis of human macrophages, T-cells and NK-cells. Enzymatic degradation and methylation studies of Vk100A2a showed that the polymer was a typical arabinogalactan pectin consisting of a highly branched rhamnogalacturonan core with approximately 50% of the rhamnose units as branch points on position 4. The side chains are composed of arabinose as terminal, 1,3-linked and 1,3,5-linked units, the galactose mainly as 1,4-linked units, but also as terminally, 1,6-, and 1,3,6-linked units. The arabinose units are mainly attached to galactose in position 3. Even after enzymatic treatment of Vk100A2a with

arabinofuranosidase and/or galactosidase, the resulting core polymer still showed high activity in the complement system suggesting that the complement fixing ability may at least in part be expressed by the carbohydrate structures present in the inner portions of Vk100A2a. The structural studies show that this polysaccharide can be classified as a RG-I type polymer with side chains both of the AG-I and AG-II types. It is interesting to note that after arabinofuranosidase treatment, the complement fixing ability is somewhat higher than for the native polymer indicating that the arabinose is modulating (reducing) the activity. The treatment involving galactanase show a reduced activity indicating that the part removed, being mainly the structural elements consisting of the 1,4-linked galactose units (AG-I) also play a positive role for the complement fixing ability [82].

4 Structure Activity Relations

As said in the introduction, this chapter focusses only on pectic type polysaccharides that have shown activity in different biological systems. The majority have shown effect on the immune system in one way or another, but a few other effects have also been observed. Table 1 gives an overview of the polysaccharide structures found for the active polysaccharides and as can be seen, a majority of the polysaccharides contain a rhamnogalacturonan I backbone. Most of these polysaccharides have attached arabinogalactan II side chains, and a few have arabinogalactan type I chains attached. Examples of pure arabinogalactan II polymers as well as rhamnogalacturonan II polymers have been found. The basic structures of all these polymers are, as described in the section "Chemical structure of pectic type polysaccharides", but as not all pectic type polymers exhibit biological activity, it is obvious that certain specific structural aspects must be present in those that show a bioactivity in the different test systems. Although structural details of the polysaccharides have been presented, only in a few cases have studies been carried out to ascertain which parts or structural details are really responsible for the bioactivity, and the group headed by Professor Haruki Yamada, Tokyo, has done most of this work.

Bupleurum falcatum pectins have been studied in great detail [46–50] as they were shown to have an effect on the complement system, anti-ulcer activity and macrophage Fc-receptor up-regulating activity to enhance immune complex clearance. The most potent fraction was the Bupleuran 2IIc that also showed a potent mitogen effect against mouse spleen cells and Peyer's patch cells of the small intestines in vitro. Detailed structural studies revealed that the ramified region contained the bioactive parts, and on this section of the molecule the oligosaccharide β-D-4-O-methyl-Glc*p*A- or β-D-Glc*p*A-1,6-β-

Table 1 An overview of the pectic type structures and bioactivities of the polysaccharides presented in this review

Plant reviewed	Type of structure				Type of activity shown	Ref.
	AG-I	AG-II	RG-I	RG-II		
Acanthus ebracteatus			×		Effect on the complement system	[29]
Angelica acutiloba	×		×		Antitumour activity	[40]
Angelica acutiloba	×	×	×		Effect on the complement system	[32–36]
Atractylodes lancea		×	×	×	Intestinal immune system modulating activity	[42–45]
Bupleurum falcatum		×	×	×	Antiulcer	[46–50]
Bupleurum falcatum		×	×	×	Effect on the complement system	[46–50]
Cistanche deserticola	×	×	×		Mitogenic and comitogenic activity	[51, 52]
Cuscuta chinensis		×	×		Immunostimulating	[53]
Cuscuta chinensis		×	×		Antioxidant	[53]
Diospyros kaki		×	×		Immunostimulating	[54]
Entada africana	×	×			Effect on the complement system	[55]
Glinus oppositifolius	×	×	×		Effect on the complement system	[56]
Glinus oppositifolius	×	×	×		Chemotactic properties towards Macrophages, T- and NK Cells	[56]
Glycyrrhiza uralensis			×		Effect on the complement system	[57]
Glycyrrhiza uralensis			×		Mitogenic activity	[57]
Glycyrrhiza uralensis			×		NK cell-mediated tumour cytotoxicity enhancer	[58]
Glycyrrhiza uralensis		×			Bone marrow cell proliferating activity	[59]
Larix spp		×			Various effects on the immunesystem and as a dietary fiber	[50–61]
Lycium barbarium	×				Immunomodulating	[62]
Melocactus depressus	×				Phagocytosis stimulating	[63]
Panax ginseng	×	×			Stimulation of production of IL-8 (Immunomodulating)	[64]

Table 1 continued

Plant reviewed	Type of structure				Type of activity shown	Ref.
	AG-I	AG-II	RG-I	RG-II		
Panax ginseng				×	Intestinal immune system modulating activity	[67–69]
Panax ginseng				×	Macrophage Fc receptor expression enhancer, IL-6 production enhancer, nerve growth factor secretion enhancer	[3, 67–69]
Panax ginseng		×	×		Effect on the complement system	[65, 66]
Piper nigrum		×			Effect on the complement system	[70]
Plantago major		×	×		Effect on the complement system	[72, 73]
Plantago major			×		Pneumococcal infection-protector	[75, 76]
Salvia officinalis			×		Mitogenic and comitogenic effect	[77]
Tinospora cordifolia			×		Activation of B-cells	[78]
Tinospora cordifolia			×		Antioxidant	[79]
Trichilia emetica		×	×		Effect on the complement system	[80]
Vernonia kotschyana	×	×	×		Effect on the complement system	[81, 82]
Vernonia kotschyana	×	×	×		Effects in different systems involved in immunomodulation	[81, 82]

D-Gal*p*-β-D-1,6-β-D-Gal*p* was shown to be a possible structural unit for the recognition of the carbohydrate receptors on the B-cells. This has not yet been published for other polysaccharides, but the group of the authors has in collaboration with Yamadas group found similar oligosaccharide structures in polysaccharides from plants that traditionally have been used against ulcers. The polyclonal antibodies prepared having affinity towards this oligomeric structure were used to show that Bupleuran 2IIc was indeed taken up by the body when given orally [48, 49].

The structure that appears to be important for the effect on the complement system is most probably the complex galactan oligomer being composed of separate 1,3- and 1,6-linked galactose chains with branch points being of 1,3,6 nature. These units have been proposed as the active sites for

this activity for the pectins from *Angelica acutiloba* [36–39], *Glinus oppositifolius* [56], *Glycyrrhiza uralensis* [26, 57, 58] and *Vernonia kotschyana* [81, 82], but they are also found in most of the polysaccharides that have an effect on the complement system. This is the so-called AG-II structure. But as not all polysaccharides containing this have an effect on the complement system, for example the larix AG-II [4], other factors related to the position of these structural units on the polymer must also be important, which has not been clarified yet. The size of these structures may be important [30] as well as the possibility that it has to have more than one binding site to be active.

Although the ramified region appears to be the most important structural feature for the effect on the complement system, it has been shown that the presence of the homogalacturonan region in the native polymers are responsible for a modulating effect, i.e. they have a down-regulating effect on the activity. This has been shown for pectins from both *Angelica acutiloba, Glycyrrhiza uralensis*, *Plantago major* and for various polysaccharides that are under study in the laboratory of the authors of this chapter. The Ara*f* units may also play a modulating role on the complement effect. It is also interesting to note that the pectic type polymer from *Acanthus ebracteatus* that is rich in 3-*O*-methyl galactose, coupled with an Mw higher than 1 mill., shows an extremely high activity compared to those pectins from the same source almost devoid of this structural feature [29].

One of the pectic fractions from *Angelica acutiloba* showed potent antitumour activity, and this polymer was rich in the AG-I type structure, indicating that the 4-linked galactose units are important for this activity [40].

Pectin polymers containing the well conserved rhamnogalacuronan II have been shown to have different types of effects in immunological test systems, but only a few of those polymers have undergone structural studies. Most of the polymers have only been characterised for the presence of some of the unusual monosaccharides that are normally found in RG-II. The one most thoroughly studied was isolated from the leaves of *Panax ginseng*, and this is according to Kiyohara [83] the only RG-II polymer that has been shown to have biological activity, and it was also shown that only the dimer form of the ginseng RG-II is bioactive [68]. How the active structural features of *P. ginseng* differ from the structural features of RG-II's isolated from other plants is not known.

Bioactivities found for some of the polysaccharides described in this chapter have been assigned to certain structural features. The antioxidant effect of the *Cuscuta chinensis* pectin was proposed to be caused by the presence of a glucobiose unit linked via a GalA unit on the RG-I polymer [53], but this structural feature was not found for the anti-oxidant polysaccharide from *Tinospora cordifolia* [78, 79].

Sufficient scientific data is still lacking to really pinpoint the bioactive sites of the pectic type polymers described in this chapter, but on the basis of the work of the group headed by Yamada over the last ten years, a better un-

derstanding of the importance of some of the structural features has been obtained. There appears to be more than one active site to accommodate all of the different activities seen, and from this review certain features have emerged as being more important than others.

5 Conclusion

From the research reviewed in this chapter it is obvious that pectins may be an important source of biologically active substances that can be used to improve the health of mankind, and especially the health of those from less favoured regions of the Earth. It is also interesting to note that most of the plants used for the isolation of the bioactive pectic type polymers reviewed have a long tradition in the use against various ailments for which the immune system may be involved in one way or another. For this reason, ethnopharmacological surveys carried out amongst people still using plants as an important part of their healthcare may be an important source for finding new bioactive plant pectins. A few surveys have been reported over the last decade. Yamada [84] has given an overview up to approx. 1995 of the contribution of pectins to healthcare. He shows that pectins have a variety of pharmacological effects, such as immunostimulating, anti-metastatic, anti-ulcer, anti-nephrosis activities, and cholesterol-reducing effects amongst others. He also describes the use of pectins in drug delivery, as adjuvant in vaccines, and also how the fine structure of each polymer is important for the activity shown of the specific polymer and concludes with observations on how pectins can be used in many ways for the benefit for human beings.

Yamadas group [85, 86] has also taken a Japanese Kampo medicine consisting of many different plants as a starting point for identifying bioactive plant polysaccharides. They found in the Kampo medicine Juzen-Taiho-To, composed of many plants, several bioactive polysaccharides with effects in different test systems that may influence the immune system. A study like this can lead to the identification of the best possible source of the plants in the mixture that contain bioactive polysaccharides.

It is not only the traditional use of a plant that may lead us to interesting new plants for bioactive pectins. From the far east, i.e. the Maritime territory and the Amur Region of Russia, Tomshich et al. [87] studied ten species from six families and found that they contained polysaccharides with possible pectic structure, and five of these plants were shown to have either immunostimulatory or antitumoural activity, or both.

Four different European herbaceous plants have also been tested for possible mitogenic and comitogenic activities. Using Zymosan as a positive control, all polysaccharide fractions tested had higher activity in the bioassays

than the Zymosan. The monosaccharide composition of the polymers investigated vary, but indicate that polysaccharides of the pectic type may be present. Further fractionation and purification of the fractions obtained may lead to the identification of pectic type polysaccharides with immunomodulatory effects [88].

Various pectins from plants traditionally used in Europe were tested for antimutagenic activity against nitroaromatic compounds [89]. Of those studied, the following pectic type polymers were found to be active: Araban from sugar beet, weakly positive; acidic pectin from apple, effective; pectin from *Cichorium sp., Citrus sp*., pectins from sugar beet were all weakly effective, while the rhamnogalacturonan from *Althaea officinalis* roots was found to be strongly effective. The latter has a rhamnogalacturanan type I structure, but is unusual as it contains side chains of glucuronic acid.

Recently, the focus has also been on wound healing plants traditionally used in the West-African country Mali. Two surveys are reported; one from the region around the capital Bamako [90], and the other from a more rural area, Dogonland [91]. The survey from the Bamako region reports the use of 123 different species belonging to 50 different plant families. Those most frequently used, were analysed for their content of polysaccharides. The monosaccharide compositions as well as their effects on the complement system were determined, and lead to the conclusion that several of the plants studied are important sources for future studies of bioactive pectic type polysaccharides. The survey from Dogonland reported 73 plants from 34 families used for wound healing that may be of interest for further investigation in order to obtain biologically active pectins.

From the science performed mainly over the last ten years it is obvious that the role of pectic substances in health care has been substantiated. For some of the pectic substances, parts of the structure of the bioactive sites have been determined, but further studies of the relevant structures for the individual active polymers must be performed in order to find a possible common structure for the activities observed. It also appears that there are special structural features present in some of the polymers, which are not found in others, and which are important for their activity, and this may explain the different behaviour of the polymers in the same system.

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Figure 4 from Carbohydrate Research, vol 338, Rodrigues-Carvajal MA, du Penhoat CH, Mazeau K, Doco T, Perez S (2003) "The three dimensional structure of the mega-oligosaccharide rhamnogalacturonan II monomer: a combined molecular modelling and NMR investigation." p 651–p671

Figure 6 from Carbohydrate Research, vol 311, Sakurai MH, Kiyohara H, Matsumoto T, Tsumuraya Y, Hashimoto Y, Yamada H (1998) "Characterization of antigenic epitopes in anti-ulcer pectic polysaccharides from Bupleurum falcatum L. using several carbohydrases." p 219–p229, all with permission from Elsevier

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