A Complement Fixing Polysaccharide from *Biophytum petersianum* Klotzsch, a Medicinal Plant from Mali, West Africa

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Biophytum petersianum Klotzsch (syn. *Biophytum sensitivum* (L.) DC) is a medicinal plant having a traditional use, among others, as a wound healing remedy in Mali and other countries. As a water extract of the aerial parts of the plant is a frequently used preparation, we decided to look for a bioactive polysaccharide in this extract. One of the obtained polysaccharide fractions, BP100 III, isolated from a 100 °C water extract from the aerial parts of *B. petersianum* and having a monosaccharide composition typical for pectic substances, was shown to exhibit potent dose-dependent complement fixating activity. The BP100 III fraction was subjected to degradation by *endo*-α-D-(1→4)-polygalacturonase, and three fractions were obtained by gel filtration. The highest molecular weight fraction, BP100 III.1, had a more potent activity in the complement test system than the native polymer, while the two lower molecular weight fractions were less active than the native polymer. The major part of BP100 III.1 consists of galacturonic acid and rhamnose, with branches being present on both the rhamnose and galacturonic acid residues. Arabinogalactan type II is also present in the polymer, indicating that BP100 III.1 has a structure typical of the hairy region of pectins. The major part of the two other fractions is a galacturonan, containing a strikingly high number of branch points, some to which xylose is attached. These results indicate that the pectic substance in *B. petersianum* contains both rhamnogalacturonan and xylogalacturonan regions.

Introduction

Biophytum petersianum Klotzsch (syn. *Biophytum sensitivum* (L.) DC) (Oxalidaceae) is a medicinal plant that has several uses in Mali and other African countries,[1] among these as a wound healing remedy. A survey on medicinal plants used for wound healing in Mali, followed by analysis of complement fixing activity, as well as the total amount and composition of carbohydrate, revealed that *B. petersianum* is a medical plant of great future interest.[2] On the basis of this report, we wanted to purify the polysaccharides from the plant and study their complement fixing activity, since several papers have demonstrated the effect of plant polysaccharides on the immune system.[3−9] In general, the complement system plays an important role as a primary defense against bacterial and viral infections and appears to be intrinsically associated with several immune reactions such as the chemotactic attraction of leucocytes, immune adherence, modulation of antibody production, and increased local vascular permeability.[10]

Several complement fixating polysaccharides, including pectins, have been isolated from bacteria, fungi, and plants, and from hot-water extracts of several medicinal plants.[4] The pectic polysaccharides are probably the most complex class of plant cell wall polysaccharides. Native pectins are believed to consist of a backbone in which "smooth" galacturonan regions of α-(1→4)-linked D-galacturonosyl residues are interrupted by ramified rhamnogalacturonan ("hairy") regions with a backbone of alternating α-(1→2)-linked L-rhamnosyl and α-(1→4)-linked D-galacturonosyl residues (rhamnogalacturonan I (RG I)). Neutral side chains are predominantly attached to O-4 of the rhamnosyl residues and composed of D-galactosyl and L-arabinosyl residues. The structure of the arabinogalactan side chains may be either of the AG type I or type II structures. AG I is basically composed of 4-linked galactose units, normally with substitutions of arabinose on position 3 of some of the galactose units, while AG II is composed of 3,6-linked galactose with chains of both 3- and 6-linked galactans that are decorated with arabinose units. The proportion of "smooth" to "hairy" regions can vary depending on the type of tissue or its development stage. Side chains of different natures, e.g., xylosyl, acetyl, apiosyl, or galactosyl units or oligomers in some cases, may also be present.[11] A minor component of the plant cell wall is rhamnogalacturonan II (RG II), which has an extremely complex structure.[12−14]

The ramified region of the pectins has, for some polysaccharides, a more potent complement-activating activity than the corresponding original pectins, while the oligogalacturonides forming the smooth regions show weak or negligible activities. These findings suggest that the complement-activating potency of pectins mainly is expressed by their ramified regions. This has been shown for pectic polysaccharides from roots of *B. falcatum* and *A. acutiloba*[4] and leaves of *P. major*[15] and *P. ginseng*.[16] The potent complement activation by the ramified region may be due to a combination of the rhamnogalacturonan core and the neutral sugar chains. Not all pectins have an effect on the complement system; the structures of pectins are highly heterogeneous, and it is believed that only a limited number of types of neutral sugar side chains in the pectins might be responsible for the expression of the activity.[4,17]

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In ref 1, *B. petersianum* is recognized as a plant traditionally used in medicine, but no studies on high molecular weight bioactive compounds present in *B. petersianum* have to our knowledge been reported. However, *Biophytum sensitivum* (L.) DC, synonym for *B. petersianum* (IPNI, W3 Tropicos, Global compendium on weeds[37]), has undergone certain investigations on the low molecular weight bioactive compounds. Different types of polyphenolic compounds, some with effects on the COX-1/COX-2 systems, have been isolated.[18−21] Insulinotropic and hypoglycemic effects were shown by an extract of *B. sensitivum* in rat,[22,23] and an antiinflammatory activity on the carrageenan-induced rat paw edema has also been shown for different types of extracts of the same plant.[24]

In this paper, we describe the isolation, structural elucidation, and biological activity of polysaccharides isolated from the medicinal plant *Biophytum petersianum* Klotzsch. The aim of the study was also to determine what part of the polysaccharide was the most important for the effect on the complement system. As described above, the presence of bioactive polysaccharides from various plants in preparations that have been previously studied may at least partly explain the wound healing effect seen in vivo and/or in vitro.

Materials and Methods

Plant Material. *Biophytum petersianum* Klotzsch, Oxalidaceae, was collected in Blendio, Mali, in 2001. The whole aerial part of the plant was used. The plant was identified by professor Drissa Diallo, Department for Traditional Medicine (DMT), Bamako, Mali, and a voucher specimen is kept at DMT.

Ethnopharmacological Studies. Healers in the regions around Sikasso, Dioila, and Kolokani in Mali were interviewed about the medicinal uses of *B. petersianum*, as well as the method of administration of the plant. The interviews were performed in the Bambara language, with professor Drissa Diallo, Department of Traditional Medicine (DMT), Bamako, and Seydou Dembelé, a plant systematician at DMT, as interpreters.

Extraction and Purification of the Polysaccharides. Powdered plant material was pre-extracted with dichloromethane (DCM), methanol (MeOH), and ethanol (EtOH) in order to remove low molecular and lipophilic substituents. The residual plant material was further extracted with 50 °C H_2O, pH 5. The aqueous extract was dialyzed against distilled water in a Spectra/Por Membrane dialysis tube (Spectrum), with molecular weight cut off of 3.5 kDa. The residual material was extracted with water at 100 °C, pH 5, and dialysed as above, and both extracts were freeze-dried. The crude extracts thus formed were purified by gel-filtration on a BioGel P2 (5 × 30 cm, Bio-Rad Laboratories, USA) column by elution with water at 1 mL/min. The eluates were monitored for the presence of carbohydrate by the phenol−sulphuric acid method[25] and the relevant fractions pooled and freeze-dried. The fractions were tested for complement fixing activity (for description of method, see below). Fraction BP100 BioGel F1, found to be the most abundant fraction, and with a high activity in the biological assay, was separated further by anion-exchange chromatography on a DEAE Sepharose fast flow column (5 × 30 cm, Pharmacia Biotech, Uppsala, Sweden) with chloride as counter ion. The column was coupled to an IKA PA-SF digital pump (IKA). The neutral polysaccharides were eluted with water at 1 mL/min and the acidic ones with a NaCl gradient (0−2 M, pH 5) at 2 mL/min. Fractions of 10 mL were collected in a Pharmacia LKB FRAC 100. The carbohydrate elution profiles were determined using the phenol sulphuric acid assay[25] and the relevant fractions pooled.

Determination of the Monosaccharide Composition of the Fractions. The monosaccharide compositions were determined by gas chromatography of the trimethylsilylated derivatives of the methyl glycosides obtained by methanolysis of the samples using 4 M HCl in anhydrous methanol at 80 °C for 24 h.[26]

Investigations for the presence of 3-deoxy-D-*manno*-2-octulosonic acid (KDO) and 3-deoxy-D-*lyxo*-2-heptulosaric acid (Dha), monosaccharides present in rhamnogalacturonan II (RG-II) type polysaccharides, were performed by the thiobarbituric acid assay (TBA assay).[27]

Enzymatic Degradation of the Polymers. Degradation with *endo*-α-D-(1→4)-polygalacturonase was performed for production of "hairy", or ramified, regions of the polymers.

A 100 mg sample was dissolved in 20 mL of 0.1 M NaOH for de-esterification and left for 2 h. The solution was neutralized by adding a few drops of acetic acid. The de-esterified sample was added to 50 mM acetate buffer, pH 4.0, and treated with Pectinase from *Aspergillus niger* (8.1 units/mg protein, EC 3.2.1.15) (Sigma, St. Louis, U.S.A.) at 30 °C. One unit of the enzymatic solution liberates 1.0 μmol of galacturonic acid from polygalacturonic acid per minute. The hydrolysis proceeded until it was complete (26 h). This was determined by the increase of reducing sugars in a reaction mixture using dinitrosalicylic acid (DNS).[28,29] The reaction was terminated by heating at 100 °C. The de-esterified and partially hydrolyzed material was fractionated by size exclusion chromatography on a BioGel P30 (2 × 88 cm, Bio-Rad Laboratories, U.S.A.) column. The column was coupled to a Peristaltic pump P-3 (Pharmacia) and a Pharmacia LKB FRAC 100, and eluted with 10 mM NaCl at 20 mL/h. The carbohydrate profile obtained was determined as above. This gave rise to three subfractions termed BP100 III.1, BP100 III.2, and BP100 III.3.

Determination of the Linkages Present in the Polysaccharide BP100 III and Subfractions. Prior to the methylation of the polymers, reduction of uronic acids to the corresponding neutral sugar has to be performed on the polymeric level.

Reduction: The uronic acids of polymer fractions were reduced to the corresponding deuterated neutral sugars with sodium borodeuteride after activation with carbodiimide.[30] The reduction step was followed by methylation and GC/MS as described below.

Methylation Analyses: Methylation of the polymers was carried out using the procedure of Sims and Bacic,[31] followed by analysis by GC-MS of the derived partially methylated alditol acetates. The gas chromatograph, Fisons GC 8065, was fitted with a split−splitless injector, used in the split mode and with a Fisons fused silica column (30 m × 0.20 mm i.d.) with film thickness of 0.20 μm. The injector temperature was 250 °C, the detector temperature 300 °C, and the column temperature 80 °C when injected, then increased by 30 °C/min to 170 °C, followed by 0.5 °C/min to 200 °C, and then 30 °C/min to 300 °C. Helium was the carrier gas with a flow rate of 0.9 mL/min. E. I. mass spectra were obtained using a Hewlett−Packard Mass Selective Detector 5970 with a Hewlett−Packard GC. The compounds at each peak was characterized by an interpretation of the retention times and the characteristic mass spectra. The estimation of the relative amounts of each linkage type was related to the total amount of each monosaccharide type as determined by methanolysis.

Molecular Weight Determination. The determination of the molecular weight of the native fraction BP100 III was determined by using SEC-MALLS as described by Hokputsa et al.[32] The molecular weight of the fractions obtained after enzymatic degradation of BP100 III followed by separation on BioGel P30 were estimated from the calibration curve of the elution volume of standard dextrans. Dextran polymers (Pharmacia) of 475, 19, 12, 6, and 2.5 kDa were used as calibration standards.

^{1}H and ^{13}C NMR. The sample was dissolved in D_2O at a concentration of approximately 0.3%. The spectra were recorded in a Bruker DRX 400 at 400.2 and 100.6 MHz, respectively, at 70 °C. The chemical shifts were expressed in parts per million relative to the resonance of the internal standard, 3-trimethylsilyl-1-propanesulfonic acid (sodium salt).

The Complement Fixation Test. The complement fixation test is based on inhibition of hemolysis of antibody-sensitized sheep erythrocytes by human sera.[33]

Briefly, sheep erythrocytes (SRBC) were washed twice with 0.9% NaCl and once with veronal buffer pH 7.2 containing 2 mg/mL bovine

serum albumin (BSA) and 0.02% sodium azid (VB/BSA) and sensitized with rabbit anti-sheep erythrocyte antibodies (Viron amboceptor 9020, Ruschlikon, Switzerland). After incubation at 37 °C for 30 min on a shaker, the cells were washed as described above, and a 1% cell suspension in VB/BSA was prepared.

The human serum used as a complement source was diluted with VB/BSA to a concentration giving about 50% hemolysis of the antibody-sensitized SRBC.

Samples dissolved in VB/BSA (50 μL) and serum dilution (50 μL) were added in duplicates to wells on a microplate and incubated on a shaker at 37 °C. After 30 min, the sensitized sheep erythrocytes (50 μL) were added and the microplate incubated as done previously. After centrifugation at 1000 g for 5 min, 100 μL of the supernatants were transferred to a flat-bottomed microplate and the absorbance at 405 nm measured using a microplate reader (Thermomax, Molecular Devices, Sunnuvale, CA). Complete 100% lysis was obtained with distilled water and sensitized sheep erythrocytes. VB/BSA, serum and sensitized sheep erythrocytes were the control of the medium, and the pectin fraction PMII from the leaves of *Plantago major* L was used as the positive control.[15] Inhibition of lysis induced by the test sample was calculated by the formula $[(A_{control} - A_{test})/A_{control}] \times 100\%$. A dose−response curve was constructed to calculate the concentration of test sample able to give 50% inihibition of lysis (ICH_{50}). Low ICH_{50} means high complement fixing activity.

Results

Ethnopharmacological Studies. In addition to the use of fresh or dried leaves from *B. petersianum* as a wound healing remedy reported by Diallo et al.,[2] a restricted survey performed by the authors showed the traditional use of the plant as a wound healer in both the villages Tarakasso and Dioila, a powder of dried aerial parts being applied to the wounds. In Sikasso and Blendio, dried aerial parts of the plant are mixed with water and drunk and/or applied to the body against malaria. Reports from several villages visited in the Kolokani region in 2004 also revealed different uses of the plant. In N'Tjbougou, a decoction of the aerial parts is used against stomachache for children, and in Didieni, a decoction of the aerial parts is used for treating malaria. In Massantola, a powder of the aerial parts is mixed with water and drunk against bad spirit, often connected to fever; fresh leaves or a powder of the aerial parts are mixed with water and drunk against malaria; a decoction of the aerial parts is used in the treatment of stomachache; and for treating fever, the body is washed using a powder of the aerial parts mixed with water. According to Burkhill,[1] the plant is used in Nigeria against stomachache, in Gabon and Zaïre as a purgative, in Ivory Coast against sores of different kinds, and it is also reported to be used against different kinds of stings and snakebites.

Isolation of Polysaccharide Fractions. The monosaccharide composition of the crude water extracts, BP 50 crude and BP100 crude, were determined and are given in Table 1. The extracts were purified by gel filtration on a BioGel P2 column; the 50 °C extract gave rise to two carbohydrate-containing fractions, while the 100 °C extract only gave one (Scheme 1). These fractions and the two crude ones were tested for complement fixing activity, and as it can be seen from Table 2, the BP 100 BioGel F1 fraction showed the most potent activity.

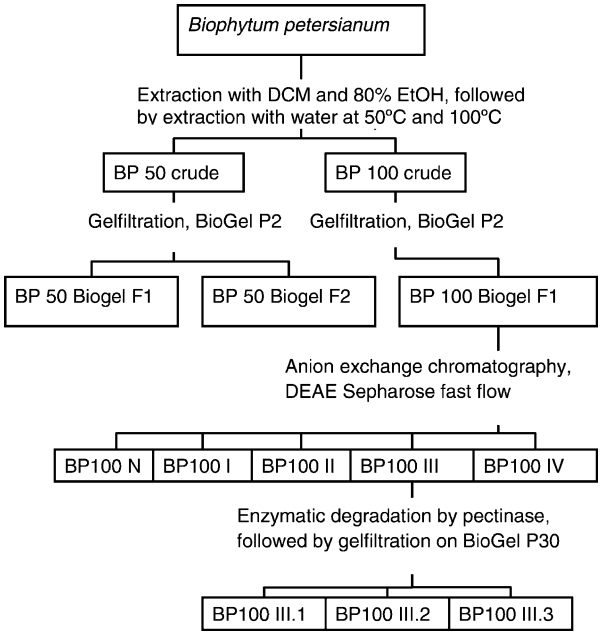
BP 100 BioGel F1 was further fractionated by ion exchange chromatography on a DEAE Sepharose fast flow column and gave one neutral and four acidic fractions that were designated BP100 N (27 mg), BP100 I (6 mg), BP100 II (8 mg), BP100 III (577 mg), and BP100 IV (87 mg). These fractions were also tested for complement fixing activity; all had an $ICH_{50} < 10$ μg/mL, as compared to that of PMII at 54 μg/mL. On the basis of the amount of material available and the results of the complement fixation test, the fraction BP100 III was chosen for further studies. The isolation procedure is given in Scheme 1.

Table 1. Monosaccharide Composition (% mol) of the Crude Water Extracts of *Biophytum petersianum*, the Major Bioactive Fraction BP100 III after Separation of the 100 °C Water Extract by Anion Exchange Chromatography, and the Fractions Obtained after Pectinase Digestion of BP100 III. MW of BP100 III and the Pectinase Degraded Fractions Are Also Given

	crude water extracts		major active fraction	fractions after pectinase degradation of BP100 III		
	50 °C	100 °C	BP100 III	BP100 III.1	BP100 III.2	BP100 III.3
Ara	12.2	21.1	8.5	7.9	11.1	10.4
Rha	10.7	8.7	7.3	22.6	12.3	7.7
Fuc	n.d.[a]	n.d.	0.8	1.1	2.3	1.9
Xyl	6.3	0.3	7.2	5.0	7.1	9.4
Man	2.3	1.0	0.4	2.0	0.8	1.0
Gal	20.0	10.9	9.0	20.0	8.5	6.4
Glc	7.2	4.9	n.d.	n.d.	n.d.	n.d.
GlcA	2.3	n.d.	n.d.	n.d.	n.d.	n.d.
GalA	38.8	53.1	64.1	38.5	55.2	60.8
total carbohydrate content (%, w/w)			51.3	92.8	92.8	102
MW			31 kD	24 kD	10 kD	8 kD

[a] n.d. = not detected.

Scheme 1. Extraction and Fractionation Scheme of *B. petersianum*[a]



[a] After Gel Filtration of the 50 °C Water Extract, BP 50 crude, two polymers were isolated, F1 being the highest molecular weight polymer and F2 the lowest. The neutral fraction after ion exchange chromatography of BP 100 Biogel F1 is labeled N, while the acidic fractions are labeled I, II, III, and IV, with I being the least acidic fraction and IV the most acidic fraction. After enzymatic degradation and gel filtration of BP100 III, the highest molecular polymer is labeled III.1 and the lower molecular weight fractions III.2 and III.3.

Monosaccharide Composition and Linkage Analyses of BP100 III. The monosaccharide composition of BP100 III is given in Table 1.

The monosaccharide composition is typical for pectins, consisting mainly of galacturonic acid (64.1%) and almost equal amounts of rhamnose, galactose, arabinose, and xylose (ap-

Table 2. Complement Fixing Activity (ICH_{50}; Concentration of the Sample at 50% Inhibition of Hemolysis) of the 50 and 100 °C Water Extracts of *Biophytum petersianum* and of Fractions Obtained by Gel Filtration on a BioGel P2 Column

extract/fraction	ICH_{50}
BP 50 crude	<7.8 μg/mL
BP 50 Biogel F1	>500 μg/mL
BP 50 Biogel F2	60 μg/mL
BP 100 crude	<7.8 μg/mL
BP 100 Biogel F1	<7.8 μg/mL
PMII[a]	60 μg/mL

[a] PMII, a pectic substance from *Plantago major* L. used as positive control.

Table 3. Linkages of BP100 III and Fractions Obtained by Gel Filtration after Pectinase Treatment (mol %)[a]

type of linkage	BP 100 III	BP100 III.1	BP100 III.2	BP100 III.3
T-Ara	6.2	7	8.5	8
1→5 Ara	2.7	1.1	0	0
T-Xyl	6.4	4.9	6.1	9.7
1→2 Xyl	0	0	1.3	0
1→4 Xyl	1.2	0.6	3.1	2.8
T-Rha	1.2	2.4	5.2	2
1→2 Rha	3.4	14.2	2.5	3
1→3 Rha	0.8	0.8	2.9	1.6
1→3,4 Rha	0.5	0	1.8	1.6
1→2,4 Rha	2.5	6.3	0.7	0.5
T-Fuc	1	1.3	2.4	2.1
T-Gal	3.1	6.8	5	1.9
1→3 Gal	2.2	4.9	3.4	3.7
1→6 Gal	1.1	3.1	0	0
1→3,6 Gal	3.3	6.2	0.6	1.4
T-GlcA	traces	traces		
T-GalA	2.5	2.3	4.1	6.4
1→4 GalA	52.3	30.2	31.2	30.1
1→3,4 GalA	8.5	6.8	17.9	22.2
1→2,4 GalA	1.1	1.1	3.3	3

[a] Determined by reduction, methylation, and GC−MS.

proximately 8%). The relatively high amount of xylose is not common in pectins, but has been observed in pectins from various plants, and is possibly a part of xylogalacturonan regions.[13] Linkage analysis was performed by methylation of the reduced polymer, followed by GC-MS of the obtained partially methylated alditol acetates. The results are given in Table 3.

The main structural feature is the 1,4-linked galacturonan, with some of the units as branch points on position 3, which is seen in certain types of pectins.[34] The rhamnose units are basically 1,2-linked, with some having branch points on position 4. The galactose and arabinose present have the normal type of linkages that are found in the arabinogalactan type II (AG II) part of certain pectins. The xylose part is mainly present as terminal units. These features have certain similarities with pectins that are composed of areas with hairy/ramified and smoother regions.[13,34]

Enzymatic Hydrolysis. The polymer BP100 III was subjected to enzymatic degradation by pectinase from *Aspergillus niger* after de-esterification by 0.1 M NaOH to isolate the hairy regions of the pectin. Pectinase hydrolyzes the smooth regions of de-esterified 1,4-linked galacturonic acid residues, leaving the hairy regions from the polymer intact, which could be isolated by gel filtration.

The pectinase hydrolysate was applied to a Bio-Gel P30 column, which resulted in three pectinase-resistant fractions,

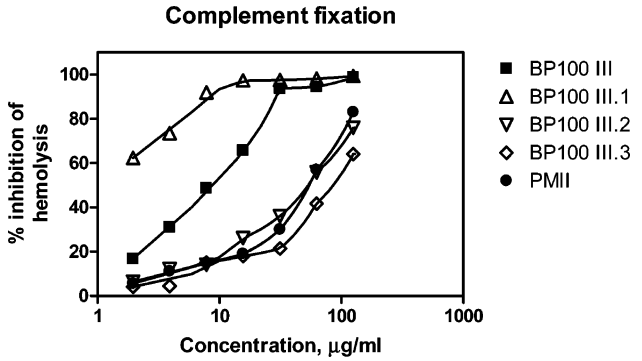


Figure 1. Dose-dependent complement fixation activity of the carbohydrate fractions obtained after pectinase degradation of BP 100 III. The PMII pectin isolated from Plantago major is used as an internal positive control. The calculated ICH_{50} was 9 μg/mL for BP100 III, <2 μg/mL for BP100 III.1, 53 μg/mL for BP100 III.2, 86 μg/mL for BP100 III.3, and 54 μg/mL for PMII.

BP100 III.1, a high molecular weight fraction, appearing in the void volume, and BP100 III.2 and BP100 III.3, with lower molecular weights. The MWs of the fractions were 24, 10, and 8 kDa, respectively, as shown in Table 1. BP100 III.1 consists of galacturonic acid (38.5%), rhamnose and galactose (appr. 20%), and equal amounts of arabinose and xylose. The two other fractions have an almost identical monosaccharide composition; the only difference is a somewhat higher amount of rhamnose in BP100 III.2 than in BP100 III.3 (Table 1). BP100 III, BP100 III.1, BP100 III.2, and BP100 III.3 all gave a negative reaction in the thiobarbituric acid assay, indicating that the monosaccharides 3-deoxy-D-*manno*-2-octulosonic acid (KDO) and 3-deoxy-D-*lyxo*-2-heptulosaric acid (Dha), and thereby rhamnogalacturonan type II (RG-II), are not present in the fractions.

Linkage Analysis of the Fractions after Enzymatic Degradation. The main feature of the highest molecular weight fraction, BP100 III.1, is the 1,4-linked galacturonic acid with a high degree of branching on position 3. Rhamnose is present as 1,2-linked units with some branch points at position 4. This latter feature is common in the hairy region of pectic substances that have side chains attached containing arabinogalactan type II polymers. These features are present in BP100 III.1. The amount of terminal xylose units almost matches the amount of branch points present on the galacturonic acid moiety, and this could be the site for these xylose units occasionally found in the hairy regions.[13,34] The other two fractions contain almost identical linkage types for the different monomers present. It is interesting to note that the high degree of branch points on the galacturonic acid units fit well with the number of terminal units found in the molecules, taking into account both the arabinose and xylose units.

Complement Fixing Activity of the Fractions after Enzymatic Degradation. The fraction BP100 III was chosen for further analyses because of the potent effect seen in the complement test system, and also because of the high amount of material available. The calculated ICH_{50} was 9 μg/mL, which is lower than that of PMII from *Plantago major*, the positive control used, which had an ICH_{50} of 54 μg/mL. The activity of the fractions obtained after pectinase degradation and separation by gel filtration showed that the highest molecular weight fraction, BP100 III.1, being equivalent to the hairy region of the native polysaccharide on the basis of the structure found, had a higher complement fixing activity (lower ICH_{50} concentration) than the native polymer (Figure 1). It is interesting to note that the two other fractions, BP100 III.2 and BP100 III.3, had more or less the same activity, and this was an activity

comparable to the positive control PMII used, an ICH_{50} of approximately 50 μg/mL. These latter activities were all lower than that of the native polymer.

NMR Results. The samples had a very low solubility, and because of this, it was difficult to obtain NMR spectra that could be used for good interpretations of the shifts that should otherwise been present. The proton spectrum showed one signal at 1.3 ppm that may be assigned to the protons on C6 of rhamnose and 1.99 ppm to the acetyl protons of possible acetyl substituents. The carbon spectrum shows weak signals at 169.1 and 21.7 ppm that may be assigned to the carbons from acetyl units, 110.1 ppm to C1 of an α-L-Ara*f* unit, and 103.4 to β-D-Gal*p* units. Signals in the region 64−77 ppm probably belong to carbons 2−5 of the monosaccharides present in the polymer. The acetyl groups were observed in aqueous solution, with no base added. Although solubilization with additional buffers with various pH values, sodium hydroxide and DMSO, was tried, no better spectra could be obtained.

Molecular Weight of BP100 III, its Hairy Region BP100 III.1, and the LMW Polymers Obtained after Enzymatic Degradation. SEC/MALLS performed under conditions when no aggregation of the molecule could take place gave a molecular weight of 31 kDa for BP100 III, but the RI trace on gel filtration showed that the fraction was polydisperse, $^1/_{20}$ of the fraction containing a polymer with a MW of 460 kDa.

The MW of the three other samples was determined by gel filtration on a BioGel P30 column. The hairy region, BP100 III.1, appeared to be relatively pure and gave a molecular weight of 24 kDa, and the other two gave 10 and 8 kDa, respectively. These results show that the hairy region of BP100 III.1, being the most active part of the molecule, represents approximately $^2/_3$ of the molecule.

Discussion

Structure Activity Relationships. The major polysaccharide fraction, BP100 III, from the 100 °C water extract of the aerial parts of *B. petersianum* was isolated after anion exchange chromatography and gel filtration. Structural studies showed that this was a pectic type polysaccharide with the features of rhamnogalacturonan 1, RG-I, type structure with side chains of the arabinogalactan type II, AG-II. In addition, the polymer also contains branches on a relatively high amount of the galacturonic acid residues, as well as terminal xylose units. This could account for a pectic structure like the ones proposed by Perez et al. and Vincken et al.[13,34] The polymer had high complement fixing activity, and after degradation by pectinase and fractionation, it was observed that the hairy region, BP100 III.1, was more active than the native polymer. This has been observed for other pectic substances as well, as reviewed by Paulsen and Barsett.[9] The two other fractions, BP100 III.2 and BP100 III.3, were also active, and surprisingly in the same range of activity as the positive control PMII. In comparison to PMII, these have a much lower MW and also a completely different carbohydrate structure. Both polymers appear to consist of a galacturonan backbone with a high degree of branching; $^1/_3$ of the galacturonan units of BP100 III.2 and 40% of BP100 III.3 are substituted with terminal arabinose and xylose units. The rhamnose present in these have, in addition to the common 1,2 linkages, a higher degree of branches on position 3 than on position 4, the latter being common for the RG-I polymers. Some of the xylose may also be present as small oligomeric chains. These features are also described in the pectin models of the Perez and Voragen groups,[13,34] but these types of structures seldom have any effect on the complement system. The hairy region usually has a backbone with a relatively high degree of branches, which also appears to be the case for the two lower molecular weight fractions isolated in this report. The presence of several binding sites is often necessary for complement activation as shown for antigen/antibody complexes[35] and is probably also valid for carbohydrate-induced complement activation. Thus, it was found that the lower MW fractions (BP100 III.2 and BP100 III.3) can have their own activities, which may act in combination to make the whole BP100 III activity lower than that of the hairy region fraction alone (BP100 III.1). The potency of the effect exerted on the complement system varying in different regions of a pectic polymer, as seen for BP100 III, has been shown for other pectic polysaccharides as well. An explanation given by Kiyohara et al.[36] is that certain areas may have a modulating effect of the activity of other areas of the total molecule, thus giving a lower activity of the originating molecule. Alternatively, the isolated hairy regions could have a more favorable exposure of the complement fixing sites than the original molecule.

Conclusion

The water extract of *Biophytum petersianum* Klotzsch, Oxalidaceae, contains polysaccharides with complement fixing activity. The main feature of the most abundant polymer, BP100 III, is that of a pectic type polymer, with hairy region (RG-I structure), some minor smooth regions, and possibly two different lower molecular weight regions which also have an effect in the complement system. When present in the native molecule, these latter might have a modulating effect on the activity of the total molecule. The complement system is associated with the wound healing process, and therefore, the traditional use of the plant as a wound healer may be connected to the complement system.

Further studies are in progress in order to further elucidate the active sites of this bioactive polysaccharide connected to the complement system, as well as in other biological assay systems.

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