STUDIES OF PLANT AND MUSHROOM POLYSACCHARIDES

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BY

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Synopsis

The present thesis entitled "**Studies of plant and mushroom polysaccharides**" is mainly aimed to determine the structure of some different polysaccharides isolated from fruits of *Psidium guajava*, seeds of *Caesalpinia bonduc*, and fruit bodies of an edible mushroom *Calocybe indica*. The entire thesis is divided into five chapters.

Chapter-1: This chapter represents the general introduction to carbohydrates, plant and mushroom polysaccharides. Carbohydrates are the first group of bioorganic compounds which are found in biological systems. They are the most abundant and diverse class of organic compounds occurring in the nature. Carbohydrates are important constituents of all living organisms, and have a variety of different functions. Some carbohydrates are the important structural components of cells and some provides the energy for life processes including growth and movement. Carbohydrates can be classified as: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. The great bulk of the carbohydrates in nature are present as polysaccharides, which have relatively large molecular weights. The polysaccharides serve two principal functions: (1) these are used by both plants and animals to store glucose as a source of future food energy, and (2) they provide some of the mechanicals structure of cells. Polysaccharides make ideal storage molecules for energy for a number of reason: a) they are large, this makes them insoluble in water and therefore they exert no osmotic or chemical effect on the cell; b) they fold into compact shapes; c) they are easily converted into the required sugars when needed.

Some carbohydrates are indigestible by humans and therefore do not provide energy (e.g., cellulose, hemicellulose, and pectin etc.). These indigestible carbohydrates form part of a group of substances known as "dietary fiber." Dietary fibers are one of the most important

components in many foods. Consumption of significant quantities of dietary fiber has been shown to be beneficial to human nutrition.

A mushroom is the fleshy, spore-bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. According to definition of Chang and Miles, "*a macro fungus with a distinctive fruiting body which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand*." Different polysaccharides from plants and mushrooms exhibit immunomodulation and antitumor properties. *Lentinus edodes* (**Lentinan, Japan**), *Schizophyllum commune* (**Schizophyllan**), *Agaricus blazei* (**Agarican, USA**), *Ganoderma lucidum* (**Lingzhi, China**), and *Grifola frondosa* (**Maitake, Japan**) have been used clinically as anti-tumor agents. The biological activities of polysaccharides depend on the size of molecule, branching rate and form. So, it is very important to determine the exact structure of the polysaccharides, isolated either from medicinal plant or from mushroom.

Chapter-2: This chapter deals with the methodologies to isolate, purify, and determine the structure of pure polysaccharides. The polysaccharides were purified using different chromatographic techniques. The exact structures of the polysaccharides were determined using two types of methods: (1) Chemical methods that include total acid hydrolysis, methylation, and periodate oxidation studies. (2) Spectroscopic methods comprising of 1D (^{1}H, ^{13}C) and 2D NMR (DQF-COSY, TOCSY, NOESY, ROESY, HMQC, HMBC etc), and mass spectroscopic (GLC-MS) experiments.

Chapter-3: *Psidium guajava* (Guava) is an important medicinal plant. The fruits show the presence of moisture (77-86%), crude fiber (2.8-5.5%), protein (0.9-1.0%), fat (0.1-0.5%), ash (0.43-0.7%), carbohydrates (9.5-10%), minerals, and vitamins, and exhibit antioxidant and hypoglycaemic properties. Natural products such as terpenoids from leaves

and flavonoids from seeds have been isolated. Extract of leaves shows antidiabetic, hypotensive, and antimicrobial effects. The hot water extract of fruits was cooled and then centrifuged. After exhaustive dialysis the filtrate was precipitated in EtOH and residue freeze dried followed by acetic acid treatment and centrifugation to yield crude polysaccharide, which on fractionation through a Sepharose 6B column in aqueous medium yielded two fractions, PS-I and PS-II. The pure polysaccharides PS-I and PS-II showed specific rotations of $[\alpha]_D^{25}$ +187.70 (c 0.068, water) and $[\alpha]_D^{28}$ +148.31 (c 0.116, water), respectively. The average molecular weights of PS-I and PS-II were estimated from a calibration curve prepared with standard dextrans as ~1.63×10^5 Da and ~1.40×10^5 Da, respectively. The structural characterization of PS-I has been presented herein.

The PS-I was hydrolyzed with 2 M CF_3COOH for 18 h at 100 °C. Paper chromatographic analysis of the hydrolyzed product showed the presence of galactose, galacturonic acid, and a slow-moving spot nearer to arabinose. The absolute configurations of the monosaccharides were determined by the method of Gerwig et al. taking intact polysaccharide and carboxyl-reduced polysaccharide. Galactose and galacturonic acid had D configuration but arabinose was present in the PS-I with L configuration. The GLC analysis of the alditol acetates of the sugars showed the presence of 2-*O*-methyl-arabinose and galactose in a molar ratio of nearly 1:1. But carboxyl-reduced polysaccharide (PS-I) on acid hydrolysis, followed by GLC analysis of the corresponding alditol acetates, showed the presence of 2-*O*-methyl-arabinose and galactose in a molar ratio of nearly 1:2. This result indicated that galacturonic acid was present in the polysaccharide (PS-I). The PS-I was then methylated using Ciucanu and Kerek method followed by hydrolysis and alditol acetate conversion. The GLC-MS analysis of alditol acetates of the methylated polysaccharide revealed the presence of 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-arabinitol and 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-D-galactitol. These results indicated the presence of (1→4)-linked-L-

arabinopyranosyl or (1→5)-linked-L-arabinofuranosyl and (1→2,4)-linked-D-galactopyranosyl moieties in a molar ratio of nearly 1:1 in the polysaccharide. The alditol acetate of methylated, carboxyl-reduced PS-I showed the peak corresponding to 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-D-galactitol along with the above peaks in a molar ratio of nearly 1:1:1. The appearance of 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-D-galactitol indicated the presence of (1→2)-linked D-galacturonic acid in the polysaccharide (PS-I). The more satisfactory result obtained from the GLC-MS analysis of alditol acetates of carboxyl-reduced methylated PS-I showed the presence of 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-arabinitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactitol, and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-D-galactitol in a molar ratio of nearly 1:1:1. This result further confirmed the presence of (1→2)-linked D-Gal*p*A in PS-I and also indicated that galactose was present as (1→4)-linked moiety along with –OAc group at 2-position. The periodate oxidation experiment was carried out with PS-I for further linking information of sugar moieties. The periodate-oxidised, reduced material of PS-I upon hydrolysis with tri-fluoro acetic acid followed by GLC analysis showed the presence of 2-*O*-methyl-arabinose and galactose. A part of periodate-oxidized PS-I on hydrolysis showed the absence of galacturonic acid in the paper chromatographic examination indicating that it had been destroyed during oxidation. GLC-MS analysis of periodate-oxidized, reduced, and methylated PS-I showed the peaks corresponding to 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-arabinitol and 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl-D-galactitol. Thus, periodate oxidation experiment confirmed that L-arabinose and D-galactose were retained while D-galacturonic acid had been destroyed.

The 500 MHz ^{1}H NMR spectrum of PS-I at 27 °C contained two signals at δ 5.06 and 4.93 for anomeric protons. The integral value of the signal at δ 5.06 was almost double to that of the other peak. Hence, the signal at δ 5.06 corresponded to two sugar residues while the

signal at δ 4.93 corresponded to one sugar residue. They were designated as **A**, **B,** and **C** according to their decreasing anomeric proton chemical shifts. In ^{13}C NMR (125 MHz) spectrum at 27 °C, three anomeric signals appeared at δ 105.3, 101.0, and 100.4. On the basis of the anomeric proton assignments, the anomeric carbon signals were readily assigned from the HMQC spectrum. Signals at δ 105.3, 101.0, and 100.4 were assigned for anomeric carbons of **A, B,** and **C** residues, respectively. All the ^{1}H and ^{13}C signals were assigned using DQF-COSY, TOCSY, HMQC, and HMBC experiments. The proton coupling constants were measured from DQF-COSY experiments.

From these chemical and NMR analysis the residues were assigned as

Residue **A**: 2-*O*-methyl-α-L-arabinosyl moity

Residue **B**: 2-*O*-acetyl-α-D-galactosyl moity

Residue **C**: methyl ester of α-D-galacturonosyl moity

The sequence of glycosyl residues of the polysaccharide was determined from NOESY as well as ROESY experiments followed by confirmation with HMBC experiment. NOESY experiment showed inter-residual contacts from **A** H-1 to **C** H-1 and **C** H-2, **B** H-1 to **A** H-4, and **C** H-1 to **B** H-4. Hence, the following sequences were established as

A **C**
→4)-2-*O*-Me-α-L-Ara*p*-(1→2)-α-D-Gal*p*A6Me-(1→

B **A**
→4)-2-*O*-Ac-α-D-Gal*p*-(1→4)-2-*O*-Me-α-L-Ara*p*-(1→

C **B**
→2)-α-D-Gal*p*A6Me-(1→4)-2-*O*-Ac-α-D-Gal*p*-(1→

The sequences in PS-I had been further confirmed from HMBC experiment. From the HMBC experiment, the cross-peaks of both anomeric proton and carbon of each of the sugar moieties were examined, and both intra- and inter-residual connectivities were observed. The inter-residual cross-peaks (**A** H-1, **C** C-2), (**A** C-1, **C** H-2); (**B** H-1, **A** C-4), (**B** C-1, **A** H-4); and (**C** H-1, **B** C-4), (**C** C-1, **B** H-4) were observed in HMBC experiment.

Based on all these chemical and spectroscopic evidences, the structure of the trisaccharide repeating unit of the polysaccharide (PS-I) was established as

A **C** **B**

→4)-2-*O*-Me-α-L-Ara*p*-(1→2)-α-D-Gal*p*A6Me-(1→4)-2-*O*-Ac-α-D-Gal*p*-(1→

Chapter-4: *Caesalpinia bonduc* (Nata Karanja) is an important medicinal plant widely distributed throughout the coastal region of India, Burma, Sri Lanka, and in other tropical and subtropical regions of the world. It is an irregular thorny shrub with large bipinnate leaves. Its flowers are yellow and fruits are inflated pods having 1-2 seeds known as nickernut. Anthelmintic activities of various extracts of leaf and fixed oil from seeds have been reported. Seed extracts showed antimicrobial, antidiabetic, antipyretic, and adaptogenic effects on animal models. The crude seed extracts, as well as a triglyceride of fatty acids isolated from seed kernel of *C. bonduc* were identified as potent antifilarial drug. Also, the methanolic extract of leaves show antitumor activity against Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. The alkaline extract of the seeds endosperm of *Caesalpinia bonduc* separated by gel-permeation chromatography yielded two fractions, PS-I and PS-II. Pure PS-I and PS-II were estimated to be 98% and 98.5% carbohydrate by colorimetric analysis, and had specific rotation $[\alpha]_D^{25}$ −16.05 (c 0.074, water) and $[\alpha]_D^{28.6}$ +21.78 (c 0.15, water), respectively. The average molecular weights of PS-I and PS-II were determined as

~2.0×10^5 Da and ~0.62×10^5 Da, respectively, compared with standard dextrans. The structural characterization of PS-I has been presented herein.

Sugar analysis of PS-I by paper chromatography and GLC of alditol acetates showed that it contained only arabinose. Configuration analysis showed that the arabinose was in the L form. Linkage analysis showed the presence of 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-arabinitol, 1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl-L-arabinitol, and 1,2,3,4,5-penta-*O*-acetyl-L-arabinitol, and thus the polysaccharide was deduced to comprise terminal non-reducing, (1→5)-linked, (1→2,5)-linked, and (1→2,3,5)-linked-L-arabinofuranosyl moieties in a molar ratio of nearly 3:2:1:1.

The 500 MHz ^{1}H NMR spectrum of the polysaccharide showed five anomeric proton signals at δ 5.23, 5.18, 5.16, 5.13, and 5.07 in a molar ratio of nearly 1:1:2:1:2. Hence, the signals at δ 5.16 and 5.07 corresponded to two residues each while the other signals at δ 5.23, 5.18, and 5.13 indicated the presence of only one residue. The sugar residues were designated as **A**-**E** according to their decreasing anomeric proton chemical shifts. In the 125 MHz ^{13}C NMR spectrum and DEPT-135 NMR spectrum four anomeric signals at δ 107.9, 107.5, 107.2, and 106.8 were present in a ratio of nearly 2:1:2:2. Signal at δ 106.8 was assigned to anomeric carbons of **A** and **B** residues, whereas signals at δ 107.2, 107.5, and 107.9 were assigned for anomeric carbons of **C**, **D,** and **E** residues, respectively. All the ^{1}H and ^{13}C signals were assigned using DQF-COSY, TOCSY, NOESY, ROESY, and HMQC experiments. The proton coupling constants were measured from DQF-COSY experiments. The very high anomeric carbon chemical shifts (δ 107.9-106.8) of all the arabinose moieties (**A**-**E**) indicated that these were present as furanose and not as a pyranose form. The coupling constants between H-1 and H-2 ($J_{H\text{-}1,\ H\text{-}2}$) of all the present L-arabinofuranosyl residues were observed in the range of 1.4-1.8 Hz indicating these were present as the α-anomer.

From these chemical and NMR analysis the residues were assigned as

Residue **A**: 2,3,5-linked α-L-arabinosyl moiety

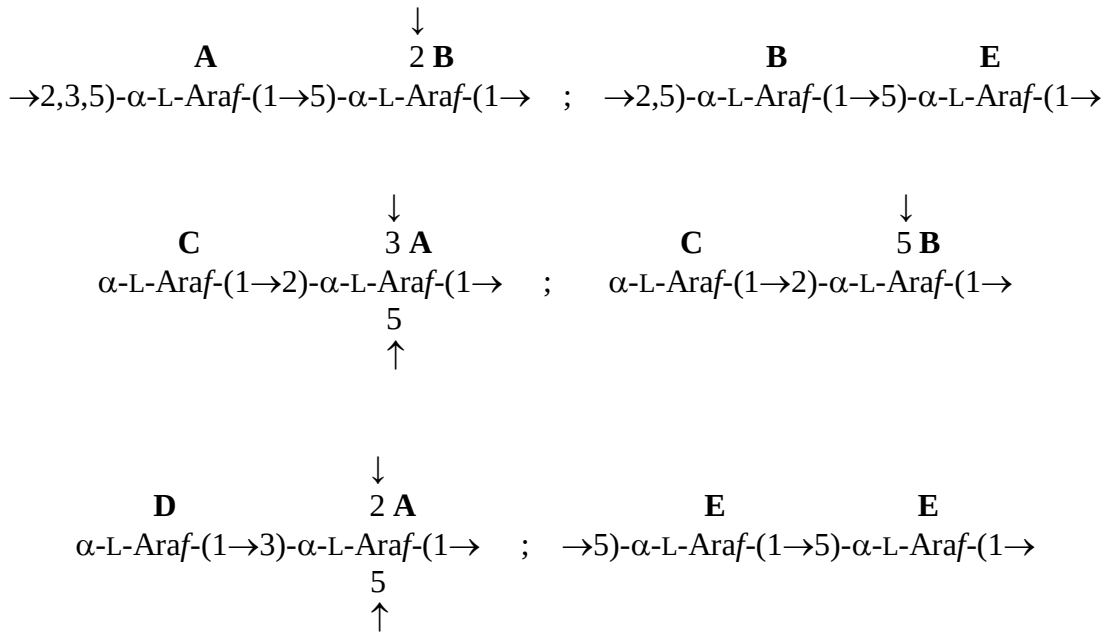
Residue **B**: 2,5-linked α-L-arabinosyl moiety

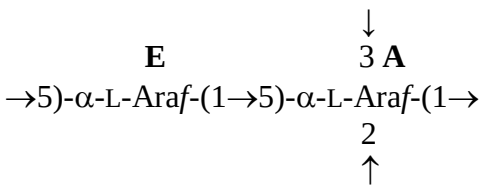
Residue **C**: terminal α-L-arabinosyl moiety

Residue **D**: terminal α-L-arabinosyl moiety

Residue **E**: 5-linked α-L-arabinosyl moiety

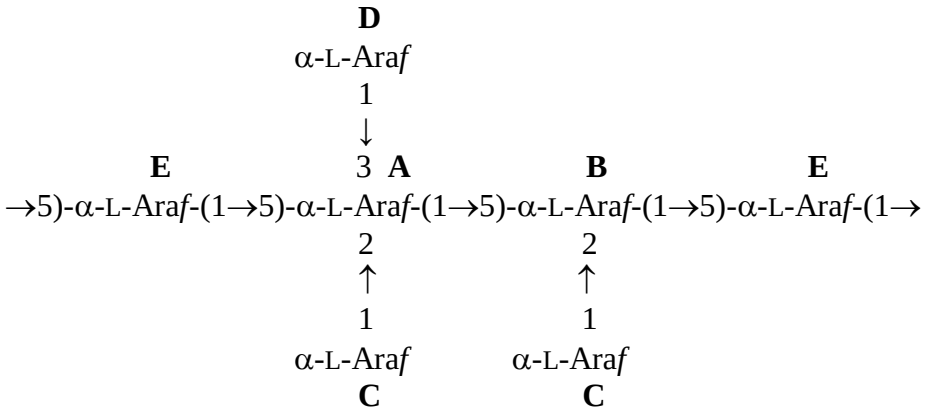
The sequence of glycosyl residues in the polysaccharide was determined from ROESY and NOESY experiments, followed by confirmation with an HMBC experiment. The ROESY experiment showed inter-residual contacts from **A** H-1 to **B** H-5a and **B** H-5b, **B** H-1 to **E** H-5a and **E** H-5b, **C** H-1 to **A** H-2 and **B** H-2, **D** H-1 to **A** H-3, **E** H-1 to **A** H-5a and **A** H-5b, and **E** H-5a and **E** H-5b of another **E** residue. Hence, the following sequences were established as





The above sequence of the glycosyl residues in the polysaccharide was further confirmed from a long-range ^{13}C-^{1}H HMBC experiment. The cross-peaks of both anomeric protons and carbons of each of the sugar moieties were examined, and both inter- and intra-residual connectivities were observed from the HMBC experiment. The inter-residual cross-peaks (**A** H-1, **B** C-5), (**A** C-1, **B** H-5a), (**A** C-1, **B** H-5b); (**B** H-1, **E** C-5), (**B** C-1, **E** H-5a), (**B** C-1, **E** H-5b); (**C** H-1, **A** C-2), (**C** C-1, **A** H-2); (**C** H-1, **B** C-2), (**C** C-1, **B** H-2); (**D** H-1, **A** C-3), (**D** C-1, **A** H-3); (**E** H-1, **A** C-5), (**E** C-1, **A** H-5b); and (**E** H-1, **E** C-5), (**E** C-1, **E** H-5a) were observed in HMBC experiment.

On the basis of all these chemical and spectroscopic evidences, the proposed structure of the heptasaccharide repeating unit of the present arabinan molecule (PS-I) was established as



Chapter-5: Mushrooms are well known for their traditional use in folk medicine, and recently for immunomodulation and anti-cancer properties. *Calocybe* genus consists of about 20 species of mushroom, including *Calocybe indica*, which is edible and can be cultivated throughout the year in the entire plains of India. It is commonly known as Dudh Chattu (milky mushroom), and a new addition to the world of edible mushrooms from India. Dudh Chattu is a robust, fleshy, milky white, umbrella like mushroom. The species is suitable for hot humid climate. *C. indica* reported to contain different types of vitamins, minerals, volatile flavored compounds, and can be cultivated using various substrates. Di-ethyl ether extract and protein fraction of *C. indica* showed antimicrobial, antiproliferative, and immunostimulatory activities. The alkaline extract of fruit bodies of *C. indica* was cooled, centrifuged, and precipitated in ethanol. The residue was dialyzed until alkali free, centrifuged, and freeze-dried to yield two new water-soluble (PS-I) and water-insoluble (PS-II) polysaccharides. The structural characterizations of these polysaccharides have been presented herein.

Structural assignment of water-soluble glucan (PS-I):

The water-soluble crude polysaccharide on fractionation through Sepharose 6B using water as an eluent yielded one homogeneous fraction. The pure water-soluble polysaccharide showed a specific rotation of $[\alpha]_D^{23.8}$ +28.07 (c 0.124, water), and the molecular weight was estimated as ~1.87×10^5 Da from a calibration curve prepared with standard dextrans. The polysaccharide was hydrolyzed with 2 M TFA. The paper chromatographic analysis and alditol acetate on GLC analysis indicated the presence of glucose only. The absolute configuration of the monosaccharide was determined by the method of Gerwig et al. as D configuration. The mode of linkages was determined by methylated using Ciucanu and Kerek method followed by hydrolysis and alditol acetate conversion. The alditol acetates of

methylated product on GLC-MS analysis showed presence of 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol, and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol in a ratio of nearly 1:2:1. These results indicated the presence of (1→4,6)-linked, (1→6)-linked, and terminal D-glucosyl moieties in the polysaccharide.

The 500 MHz ^{1}H NMR spectrum contained three signals at δ 5.38, 4.52, and 4.50 for anomeric protons in a ratio of nearly 1:1:2. They were designated as **A**, **B,** and **C** according to their decreasing anomeric proton chemical shifts. In ^{13}C and DEPT-135 NMR (125 MHz) spectrum at 27 °C, three anomeric signals appeared at δ 103.4, 103.2, and 100.0 in a ratio of nearly 2:1:1. Based on the result of the HMQC experiment, the anomeric carbon signals at δ 100.0, 103.2, and 103.4 ppm were corresponded to the anomeric proton signals at δ 5.38, 4.52, and 4.50 ppm, respectively. These data indicated that the proton signal at δ 4.50 corresponded to two sugar residue, while the others corresponded for one. All the ^{1}H and ^{13}C signals were assigned using DQF-COSY, TOCSY, ROESY, NOESY, and HMQC experiments. The proton coupling constants and C-1, H-1 coupling constants were measured from DQF-COSY and proton coupled ^{13}C NMR experiment, respectively.

From these chemical and NMR analysis the residues were assigned as

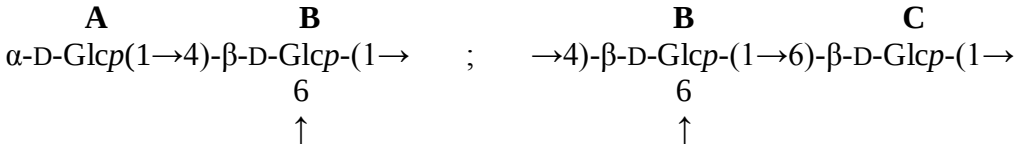
Residue **A**: α- D-glucosyl moiety

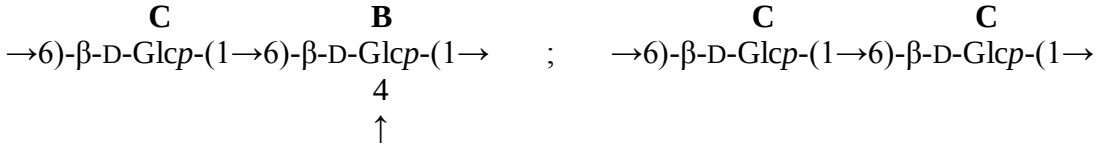
Residue **B**: β-D-glucosyl moiety

Residue **C**: β-D-glucosyl moiety

The sequence of glycosyl residues of the polysaccharide was determined from NOESY as well as ROESY experiments followed by confirmation with HMBC experiment. NOESY

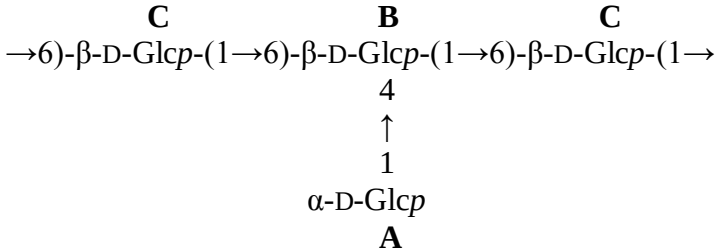
experiment showed inter-residual contacts from **A** H-1 to **B** H-4; **B** H-1 to **C** H-6a and **C** H-6b; **C** H-1 to **B** H-6a and **B** H-6b, and **C** H-6a and **C** H-6b of another **C** residue. Hence, the following sequences were established as





A long-range ^{13}C-^{1}H HMBC experiment further confirmed the above-mentioned sequences deduced from NOESY experiment. From the HMBC experiment, the cross-peaks of both anomeric proton and carbon of each of the sugar moieties were examined, and both intra- and inter-residual connectivities were observed. The inter-residual cross-peaks (**A** H-1, **B** C-4), (**A** C-1, **B** H-4); (**B** H-1, **C** C-6), (**B** C-1, **C** H-6a and **C** H-6b); (**C** H-1, **B** C-6), (**C** C-1, **B** H-6a and **B** H-6b); (**C** H-1, **C** C-6), (**C** C-1, **C** H-6a and **C** H-6b) were observed in HMBC experiment.

Based on all these chemical and spectroscopic evidences, the structure of the tetrasaccharide repeating unit of the water-soluble glucan was established as



(PS-I: water-soluble)

Structural assignment of water-insoluble glucan (PS-II), *Calocyban*:

The molecular weight of the water-insoluble glucan (PS-II), *Calocyban* was estimated from a calibration curve prepared with standard dextrans as ~2.0×10^5 Da. The polysaccharide was hydrolyzed by 2 M trifluoroacetic acid (TFA), and the alditol acetates of the hydrolyzate were analyzed by GLC using columns A (3% ECNSS M) and B (1% OV-225). The analysis showed the presence of glucose, only. Paper chromatographic analysis of the hydrolyzate further confirmed the presence of glucose. The absolute configuration of the monosaccharide was determined as D configuration by the method of Gerwig et al. For assigning the mode of linkages, the glucan was methylated by the method of Ciucanu and Kerek, followed by hydrolysis and alditol acetate preparation. The alditol acetates were analyzed through GLC using columns A and B, and also by GLC-MS analysis performed on Shimadzu GC-MS Model QP2010 Plus automatic system, using ZB-5MS capillary column (30 m × 0.25 mm), revealed the presence of 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-glucitol, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-glucitol, and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol in a ratio of nearly 1:3:1. These results indicated the presence of (1→3,4)-linked, (1→3)-linked, and terminal D-glucosyl moieties in the glucan. For further linking information of sugar moieties, the periodate oxidation experiment was carried out with the glucan. GLC-MS analysis of the alditol acetates of the periodate-oxidized, $NaBH_4$ reduced, methylated polysaccharide showed the presence of 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-glucitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-glucitol in a ratio of nearly 1:3. This result indicated that (1→3,4)-linked and (1→3)-linked residues were retained, while the non-reducing terminal D-glucosyl moiety was destroyed during oxidation.

In the 125 MHz ^{13}C NMR spectrum at 27 °C, the anomeric carbon signal of all the residues at δ 103.9 ppm was the clear evidence of β-conformation of the D-glycosyl residues.

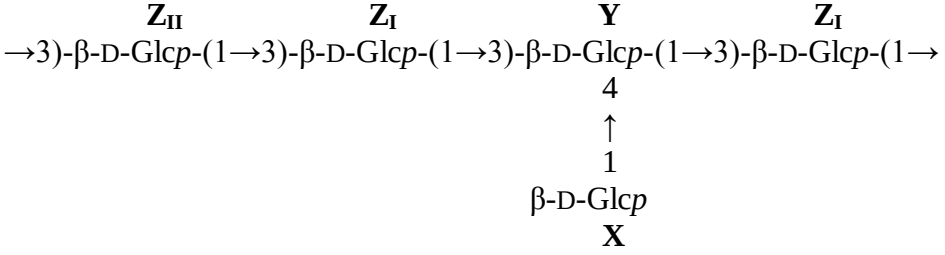
DEPT-135 NMR spectra showed the absence of C-6 linkage in PS-II. The non-reducing terminal D-glucosyl, (1→3,4)-, and (1→3)-linked moieties were designated as **X**, **Y**, and **Z**, respectively.

Residue **X**: terminal β-D-glucosyl moiety

Residue **Y**: 3,4-linked β-D-glucosyl moiety

Residue **Z**: 3-linked β-D-glucosyl moiety

The (1→3)-linked residues were designated as **Z**. Among the (1→3)-linked residues, two $\mathbf{Z_I}$ residues were situated adjacent to the residue **Y**, and one $\mathbf{Z_{II}}$ residue was away from it. C-3 (δ 87.3) of $\mathbf{Z_I}$ showed upfield shift due to neighboring effect of the most rigid part of the backbone '**Y**' while C-3 (δ 87.7) of $\mathbf{Z_{II}}$ reasonably appeared in downfield region. Hence, it is a branched (1→3)-, (1→4)-β-D-glucan consisting of a pentasaccharide segment with one non-reducing terminal β-glucosyl residue and four internal β-glucosyl residues, of which one is 3,4-disubstituted and three are 3-mono substituted. Therefore, based on all the above chemical and spectroscopic evidences, the possible structure of the pentasaccharide repeating unit of the water-insoluble β-glucan was established as



(PS-II: water-insoluble glucan, *Calocyban*)