Carbohydrate Research, 168 (1987) 55–65
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

ISOLATION AND CHARACTERIZATION OF β-D-GLUCAN, HETEROPOLYSACCHARIDE, AND TREHALOSE COMPONENTS OF THE BASIDIOMYCETOUS LICHEN *Cora pavonia**

MARCELLO IACOMINI, SANDRA M. W. ZANIN, JOSÉ D. FONTANA,
Departamento de Bioquímica, Universidade Federal do Paraná, C. Postal 19046, 81.504 Curitiba, PR (Brazil)

LAWRENCE HOGGE, AND PHILIP A. J. GORIN†
Plant Biotechnology Institute, National Research Council, Saskatoon, Saskatchewan S7N OW9 (Canada)

(Received August 2nd 1985; accepted for publication in revised form, August 11th, 1986)

ABSTRACT

Cora pavonia, a lichen having a basidiomycetous mycobiont, is rich in protein (36%), of which 10% is tyrosine. α,α-Trehalose is present and was isolated in 4.4% yield. The lichen was found to contain polysaccharides typical of basidiomycetes and different from those of ascomycetes and ascomycetous lichens. Isolated and characterized were a β-D-glucan and a heteropolysaccharide containing L-rhamnose, L-fucose, D-xylose, D-mannose, D-glucose, and D-galactose. The β-D-glucan was highly branched with 21% of nonreducing end-groups, contained 3-*O*-, 6-*O*-, and 3,6-di-*O*-substituted β-D-glucopyranosyl units, and had a main chain consisting of (1→3)- and (1→6)-links. The heteropolysaccharide component contained mainly mannose and xylose, having a mannose-containing nucleus and a main chain with preponderant (1→3)-linked α-D-mannopyranosyl residues. These were unsubstituted (10%), and 4-*O*- (10%) and 2,4-di-*O*-substituted (10%) with residues of β-D-xylopyranose. On methylation analysis of the heteropolysaccharide, a capillary column of DB-210 proved to be particularly useful for gas–liquid chromatographic resolution of partially *O*-methylated alditol acetates.

INTRODUCTION

Cora pavonia, known commonly in Brazil as "asa de papagaio" (parrot's wing), is one of only a few lichens having a basidiomycetous rather than an ascomycetous mycosymbiont, and has never been subjected to a chemical analysis. In

*NRC No. 24320.
†Present address: Departamento de Bioquímica, Universidade Federal do Paraná, C. Postal 19046, 81.504 Curitiba, PR (Brazil).

0008-6215/87/$ 03.50

TABLE I

AMINO ACID COMPOSITION OF *Cora pavonia*[a]

Amino acid	*Molar comp. (%)*	*Amino acid*	*Molar comp. (%)*	*Amino acid*	*Molar comp. (%)*
Tryptophan	0.95	Serine	4.6	Methionine	1.3
Lysine	5.0	Glutamic acid	7.9	Isoleucine	4.3
Histidine	2.1	Proline	3.8	Leucine	6.4
Ammonia	1.6	Glycine	4.3	Tyrosine	10.1
Arginine	4.8	Alanine	5.1	Phenylalanine	5.3
Aspartic acid	8.8	Half cystine	1.6		
Threonine	4.7	Valine	5.7		

[a]Protein content, 36.05%.

the present study, the protein and carbohydrate components were investigated and compared with counterparts of ascomycetous lichens.

RESULTS AND DISCUSSION

C. pavonia contains 36% of protein, which is unusually high when compared with 4–20% reported for ascomycetous lichens[1]. Amino acid analysis (Table I) revealed a high value (10%) for tyrosine which constitutes only 4–7% of the total amino acids in ascomycetous lichens[1].

Hot benzene–ethanol extraction of *C. pavonia* removed nonpolar material which was discarded and was followed by treatment of the residue with refluxing 80% aqueous methanol, which solubilized low-molecular-weight carbohydrates. After de-ionization, the extract gave, on paper chromatograms, a major spot having R_{Gal} 0.4 (4.4% yield) and a trace of mannitol (0.13% yield, as determined by g.l.c. of acetate). Hydrolysis of the material gave glucose and a compound appearing to be α,α-trehalose, as it had a strong, positive specific rotation and its ^{13}C-n.m.r. spectrum contained six signals with typical chemical shifts. The material was then isolated in crystalline form and the identification confirmed.

Lichen, previously treated with benzene–ethanol and aqueous methanol, was subjected to two procedures in order to isolate the polysaccharide components. One of them was a hot aqueous extraction, followed by partial evaporation, freezing, and gentle thawing. This is a standard procedure in the case of ascomycetous lichens because an insoluble glucan can often be isolated. In the present experiment, however, the precipitate was noncarbohydrate. Carbohydrate in the supernatant represented 4.7% of the original lichen and consisted of polysaccharide containing rhamnose, fucose, xylose, mannose, galactose, and glucose in a ratio of 5:12:22:19:15:27. The polysaccharide was subjected to fractionations with Fehling solution giving rise to a heteropolysaccharide, isolated *via* its insoluble copper complex, and glucan, obtained from the supernatant. It was necessary to carry out four

successive precipitations of the heteropolysaccharide (0.06% yield) with Fehling solution in order to obtain a sample having a constant ratio of rhamnose, fucose xylose, mannose, galactose, and glucose (4:10:32:29:17:8). Similarly, four treatments were needed to remove heteropolysaccharide from the glucan, *via* Fehling precipitation, before a pure sample was obtained (0.04% yield). The ^{13}C-n.m.r. spectra of glucan and heteropolysaccharide are depicted in Fig. 1A and Fig. 1B, respectively.

The other extraction procedure involved treatment with hot aqueous potassium hydroxide, followed by neutralization with acetic acid, and isolation of material (15% yield) *via* ethanol precipitation. Inositol was detected in the supernatant. The precipitate contained rhamnose, fucose, xylose, mannose, galactose, and glucose in a 5:9:23:21:13:28 ratio, and one Fehling fractionation procedure, as described above, gave a glucan (1.4% yield) and a heteropolysaccharide (0.18% yield), the latter having a sugar composition close (3:13:36:32:11:5) to that of the product isolated by aqueous extraction. The two different extraction procedures gave virtually identical glucans and heteropolysaccharides according to the similar ^{13}C-n.m.r. spectra (Figs. 1A and 1B, respectively). On the basis of this result and since, after three more fractionations of each with Fehling solution, the yields of glucan and heteropolysaccharide were similar to those obtained from the aqueous extract, only the products arising from aqueous extraction were further examined. Unlike the fractionation by the Fehling procedure, only a partial fractionation was obtained by use of column chromatography on DEAE-cellulose, which gave mixed polysaccharides on elution with 0.1M phosphate buffer (pH 6.8) and pure glucan on

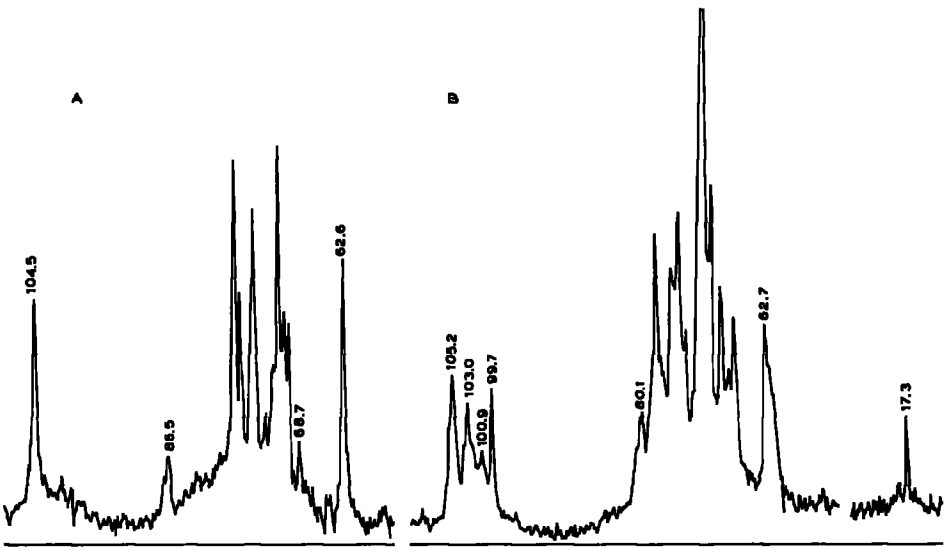


Fig. 1. ^{13}C-n.m.r. spectra of *C. pavonia* β-D-glucan (A) and heteropolysaccharide (B). The solvent was D_2O at 70° and the numerical values (δ) are based on the signal of tetramethylsilane obtained in a separate experiment.

washing with 0.3M sodium hydroxide. No fractional precipitation was obtained with Cetavlon in the presence of borate ion and variation of the pH.

The glucan component obtained after aqueous extraction was homogeneous on ultracentrifugation (1.4 *S*), being free of the heteropolysaccharide having 2.6 *S* (see below). It showed a specific rotation ($[\alpha]_D^{25}$ +13°) consistent with a β-D-glucopyranan structure. Its ^{13}C-n.m.r. spectrum (Fig. 1A) resembled those of the glucans obtained from the fungi *Schizophyllum commune* and *Lentinus edodes*, suggesting a branched structure with (1→3)- and (1→6)-linked β-D-glucopyranosyl residues[2]. The spectrum showed a low-field, C-1 signal corresponding to β-D-linked units[3] and another at δ 86.5 arising from 3-*O*-substituted residues of β-D-glucopyranose[2,4]. Methylation analysis of the *C. pavonia* glucan furnished partially *O*-methylated alditol acetates which were examined by g.l.c.–m.s. in a capillary column coated with 1:3 OV-225–OV-17, which resolves tri-*O*-methyl derivatives[5]. Nonreducing end-groups (21%), and 3-*O*- (30%), 6-*O*- (30%), and 3,6-di-*O*-substituted (19%) units of glucopyranose were characterized. The glucan was subjected to a Smith degradation incorporating mild hydrolytic conditions and the product, when examined on a paper chromatogram, showed mainly a spot for glycerol and a series of slower-moving spots whose mobilities suggested oligosaccharide structures. The presence of these compounds and the absence of a polymeric product precipitable with ethanol indicated that the glucan does not have a (1→3)-linked β-D-glucopyranosyl main-chain as have many fungal β-D-glucans[6]. Instead, the (1→3)-linkages are interspersed with (1→6)-linkages as in the glucans of *Piricularia oryzae* and *L. edodes*[7].

The heteropolysaccharide of the aqueous extract was subjected to various tests which demonstrated its homogeneity. On ultracentrifugation, it was found to be homogeneous but polydisperse with 2.6 *S*, and was free of the D-glucan with 1.4 *S*. Elution from Sepharose Gel 4B-200 with water gave a single band. Both electrophoreses on Cellogel in aqueous barbital (pH 8.6) or sodium borate (pH 8.6) gave a single carbohydrate and a weakly positive protein band in the same position. The heteropolysaccharide contained 5.1% of protein, which could be acidic and account for its mobility in the barbital buffer. However, its ^{13}C-n.m.r. spectrum did not contain a signal at δ 175 corresponding to a carboxyl group.

On hydrolysis, the heteropolysaccharide gave rhamnose, fucose, xylose, mannose, galactose, and glucose. Fucose was in the L form as it was oxidized by L-fucose dehydrogenase. Similarly, D-galactose dehydrogenase oxidized the galactose component, showing that it has the D-form. The hydrolyzate was fractionated by cellulose-column chromatography, and glucose, galactose, and mannose were each isolated along with mixed fractions, one containing rhamnose and fucose, and the other fucose and xylose. Each component was converted into its (−)-2-octyl glycoside acetate derivative and examined by capillary g.l.c. (Durowax-4) which identified the D- or L form[8]. Thus, L-rhamnose, L-fucose, D-xylose, D-mannose, D-galactose, and D-glucose, were observed.

A Smith degradation of the heteropolysaccharide incorporating mild hydroly-

TABLE II

G.L.C. ANALYSIS OF PARTIALLY *O*-METHYLATED ALDITOL ACETATES OBTAINED FROM METHYLATED, MANNOSE-RICH POLYSACCHARIDE DERIVED BY SMITH DEGRADATION FROM HETEROPOLYSACCHARIDE[a]

Alditol	T_M[b]	*Proportion (%)*[c]
2,3,4,6-Me_4-Man	1.00	6
2,3,4,6-Me_4-Glc	1.10	12
2,4,6-Me_3-Man	1.48	57
2,4,6-Me_3-Glc	1.51	12
2,4,6-Me_3-Gal	1.52	6
2,3,6-Me_3-Glc	1.80	7
4,6-Me_2-Man	2.30	5

[a]DB-210 capillary column. [b]Retention time (T_M) relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol. [c]Percentage of total peaks obtained from methylated polysaccharide.

tic conditions was carried out. The products consisted of glycerol, a tetritol, and an ethanol-insoluble polysaccharide having $[\alpha]_D^{25}$ +93°. This contained rhamnose, fucose, xylose, mannose, galactose, and glucose in a 2:1:2:80:9:6 ratio, suggesting an α-D-mannopyranosyl core. Since the original polysaccharide had a lower specific rotation of $[\alpha]_D^{25}$ +25°, the units of the other major component (D-xylose) that were eliminated by oxidation, had the β configuration. It is likely that β-D-xylopyranosyl units gave rise, in the ^{13}C-n.m.r. spectrum, to the strong C-1 signal at δ 105.2 (Fig. 1B). The polysaccharide formed by Smith degradation of the heteropolysaccharide was subjected to methylation analysis and the resulting *O*-methyl alditol acetates were analyzed by g.l.c.–m.s. using a capillary column of DB-210. The principal peak showed that 57% of 3-*O*-substituted mannopyranosyl units were present and should comprise part of the main chain. Nonreducing end-groups (6%) and 2,3-di-*O*-substituted units (5%) of mannopyranose were also present and Table II shows that the total proportion of mannopyranosyl derivatives recovered (68%) does not correspond to the proportion of mannopyranosyl residues (80%) in the original, Smith-degraded polysaccharide. It is likely that the mannose-containing component had a molecular-weight lower than those of other components and preferentially migrated through the dialysis tube during the Haworth methylation procedure. A more accurate indication of the high proportion of (1→3)-linkages was obtained on periodate oxidation of the polysaccharide, which resulted in the consumption of 0.09 mol of oxidant/unit mol, after 18 h, with formation of 0.04 mol of formic acid/unit mol.

Methylation analysis of the heteropolysaccharide was difficult to interpret as many *O*-methylalditol acetates were formed. A preliminary g.l.c.–m.s. experiment, carried out in a capillary column of 3:1 OV-225–OV-17, gave 13 peaks. However, the preferred column was that containing DB-210 as 22 peaks were obtained (see Table III). It could also resolve mixtures of derivatives that might be formed in the reaction, namely, (a) the acetates of 6-*O*-methyl derivatives of mannitol, glucitol, and galactitol, (b) the acetates of 2,6-di-*O*-methyl derivatives of mannitol, glucitol,

TABLE III

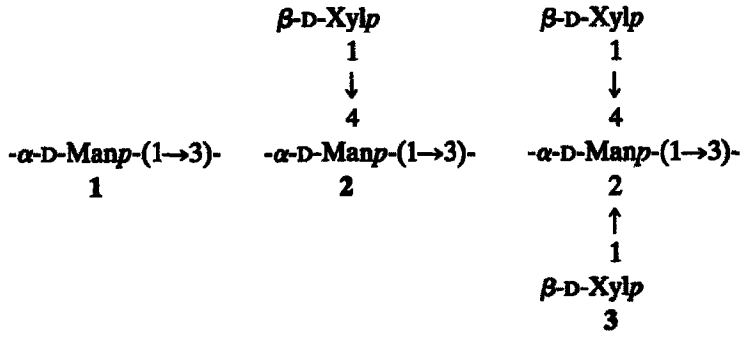
G.L.C. ANALYSIS OF PARTIALLY *O*-METHYLATED ALDITOL ACETATES OBTAINED FROM HETEROPOLYSACCHARIDE[a]

Alditol	T_M[b]	*Proportion (%)*	*Alditol*	T_M[b]	*Proportion (%)*
2,3,4-Me_3-Rha	0.73	1	2,4,6-Me_2-Glc	1.51	2
2,3,4-Me_3-Xyl	0.82	25	2,4,6-Me_3-Gal	1.52	2
2,3,4-Me_3-Fuc	0.84	6	3,4,6-Me_3-Man	1.63	1
2,3,4,6-Me_4-Man	1.00	4	2,3,6-Me_3-Man	1.67	2
2,3,4,6-Me_4Gal	1.01	2	2,3,6-Me_3-Gal	1.80	2
2,4-Me_2-Rha	1.09	1	2,3,6-Me_3-Glc	1.85	3
2,3,4,6-Me_4-Gal	1.10	2	2,3,4-Me_3-Man	1.89	2
2,4-Me_2-Fuc	1.13	1	2,3,4-Me_3-Gal	2.26	9
3,4-Me_2-Rha(Fuc)	1.15	1	2,6-Me_2-Man	2.31	10
2,4-Me_2-Xyl	1.19	2	6-Me-Man	2.98	10
2,4,6-Me_3-Man	1.48	10	3,4-Me_2-Gal	3.92	2

[a,b,c]See footnotes to Table II.

and galactitol, and (c) the acetates of 3,4-di-*O*-methyl derivatives of mannitol and galactitol. On periodate oxidation, the heteropolysaccharide consumed, after 72 h, 0.92 mol of oxidant/unit mol with formation of 0.43 mol of formic acid/unit mol. The latter value is close to that expected from the methylation data (Table III), which indicates 40% of nonreducing end-groups and 11% of 6-*O*-substituted residues. G.l.c. on 3:1 OV-225–OV-17 showed the presence of 7% of 2,3-di-*O*-methylxylitol acetate derived from methylated polysaccharide. Since this result could signify the presence of 2-*O*- or 4-*O*-substitution, the polysaccharide was degraded into a mixture of *O*-methylaldoses that were converted into methyl glycosides. G.l.c. analysis on DB-210 showed a peak corresponding to methyl 3,4-di-*O*-methylxylopyranoside but not the 2,3-isomer, thus indicating 2-*O*-substitution. As g.l.c. on DB-210 gave a poor resolution of the 2,4,6-derivatives of mannitol, glucitol, and galactitol, as alditol acetates, the separation was performed on a capillary column of OV-17, which is known to resolve this mixture[9], and a ratio of 15:5:63:18 was obtained for the acetates of 2,4,6-*O*-methylgalactitol, 2,3,6-tri-*O*-methylglucitol, 2,4,6-tri-*O*-methylmannitol, and 2,4,6-tri-*O*-methylglucitol.

Some structures having a main chain composed principally of (1→3)-linked α-D-mannopyranosyl units may be proposed for the heteropolysaccharide on the basis of the Smith degradation and the methylation data (Table III). The main chain is unsubstituted (**1**; 10%), 4-*O*-substituted with β-D-xylopyranosyl groups



(2; 10%), and 2,4-di-*O*-substituted with β-D-xylopyranosyl groups (3; 10%). Other 2-*O*-substituted xylopyranosyl residues (~7%) are a likely part of the side chains.

The polysaccharide components of *C. pavonia*, a β-D-glucan and a heteropolysaccharide, have structures typical for basidiomycetes and probably arise from the mycobiont rather than the phycobiont portion of the lichen. The β-D-glucan has (1→3)- and (1→6)-linkages and is highly branched with 21% of non-reducing end-groups. Thus, it resembles D-glucans of basidiomycetes rather than those of ascomycetes, which have relatively few branches (10% or less). In ascomycetous lichens, the β-D-glucans are linear with either (1→6)- or a mixture of (1→3)- and (1→4)-linkages[6]. The heteropolysaccharide of *C. pavonia* has a main chain consisting of a high proportion of (1→3)-linked α-D-mannopyranosyl units, which is more typical of basidiomycetes rather than of ascomycetes[6] and ascomycetous lichens[10], which mostly contain mannose-containing polysaccharides having (1→6)-linked α-D-mannopyranosyl main-chains. *C. pavonia* contains a surprisingly high proportion (4.4%) of α,α-trehalose. This sugar, however, is not exclusively characteristic of basidiomycetes, as it is also present in the ascomycetous lichens, *Umbilicaria pustulata*[11] and *Dermatocarpon minitiatum*[12], and a wide range of cells and tissues. These include plants, algae, fungi and yeasts, bacteria, insects, and other invertebrates[13]. For the preparation of α,α-trehalose, various yields of 2% (ref. 14) and 10–15% (ref. 15) have been reported when dried, pressed yeast is used as a source. α,α-Trehalose is present in trehala manna[16] (25–30%), and the resurrection plant *(Selaginella lepidophylla)* of the American Southwest, with its 2% content, has been suggested as a commercial source[17].

EXPERIMENTAL

Collection of C. pavonia. — The lichen was found mixed with mosses along stretches of man-made roadside banks of the highway Estrada Graciosa, Paraná, Brazil. The exact locale is 1 km from the post of Polícia Florestal, in the direction of Morretes, at an altitude of 900 m and is situated on the land side of the height of land.

Isolation of α,α-trehalose from C. pavonia. — Dried lichen (150 g) was ex-

tracted twice with 9:1 (v/v) benzene–ethanol (1.5 L) at 60–70° for 3 h, and then by refluxing in 80% aqueous methanol (1 L) for 3 h. The latter extract was evaporated to dryness, the residue dissolved in water, and the solution treated with mixed-bed, ion-exchange resins. Filtration followed by evaporation of the filtrate provided a residue (6.7 g, 4.4% yield) which gave a major spot on a paper chromatogram in 1:5:3:3 (v/v) benzene–butanol–pyridine–water (spray: $AgNO_3$–NH_4OH), R_{Gal} 0.4; ^{13}C-n.m.r.: δ 62.4, 71.5, 72.8, 73.8, 74.3, and 95.1, corresponding to those of α,α-trehalose. Crystallization from water–methanol–ethanol gave material, m.p. 97°, $[\alpha]_D^{25}$ +174° (*c* 0.5, water); lit.[18] (dihydrate) m.p. 97°, $[\alpha]_D^{18}$ +178°.

Anal. Calc. for $C_{12}H_{26}O_{13}$: C, 38.09; H, 6.93. Found: C, 38.16; H, 6.53.

Mannitol (0.13%) was detected by g.l.c. of acetates derived from the de-ionized preparation just described.

Isolation of polysaccharide from C. pavonia. — Lichen (150 g) that had been previously extracted with benzene–ethanol and aqueous methanol was treated with water (2 L) at 100° for 8 h. The lichen was filtered off while hot and the extraction process repeated 5 times. The combined filtrates were frozen, thawed at ambient temperature, and the insoluble, noncarbohydrate material which formed was centrifuged off. The supernatant solution (1 L) was concentrated to 200 mL and addition to excess ethanol precipitated a polysaccharide (5.5 g) containing rhamnose, fucose, xylose, mannose, galactose, and glucose in a ratio of 5:12:22:19:15:27.

The polysaccharide was dissolved in water and to the solution was added Fehling solution (200 mL). The precipitate was filtered off and washed successively with cold 2% aqueous KOH and methanol. The copper complex was decomposed by shaking with an aqueous suspension of Amberlite IR-120 (H^+) which was filtered off. The filtrate was concentrated to a small volume, and addition to excess ethanol precipitated a heteropolysaccharide. This was isolated, redissolved in water, and precipitated with Fehling solution. The process was repeated twice more until the sugar composition was constant (5.1% protein also). On ultracentrifugation of a 0.45% solution in 0.1M NaOH, the heteropolysaccharide had 2.6 *S*. It was eluted as a single band by water from a column of Sepharose 4B-200 at the same rate as Sigma Blue Dextran (mol. wt. 2×10^6). It was homogeneous on electrophoresis on Cellogel in 0.4M barbital buffer, pH 8.6, and in 0.05M sodium borate, pH 8.6, giving a single carbohydrate (Schiff-periodate[19]) and protein-positive (Coomassie Brilliant blue[20]) band.

The supernatant solution of the first Fehling precipitation was made neutral with acetic acid, dialyzed against tap water for 3 days, and then de-ionized with resins. The solution was concentrated to a small volume, added to excess ethanol, and the precipitated polysaccharide centrifuged off. This preparation was still contaminated with heteropolysaccharide as the treatment of an aqueous solution with Fehling solution gave a precipitate. This was removed, the D-glucan isolated, and the process repeated twice to completely remove impurities. Electrophoresis in borate buffer, pH 8.6, showed a single band which was carbohydrate-positive but was barely perceptible with the Coomassie Blue reagent.

In another extraction procedure, lichen (100 g) was treated successively with benzene–ethanol, aqueous methanol, and then with 2% aqueous KOH (1 L) at 100° for 2 h. The mixture was made neutral with acetic acid and filtered, and the filtrate concentrated to a small volume and added to excess ethanol. The precipitate was isolated and, since it was found to contain inositol liberated on treatment with alkali, it was redissolved in water and the precipitation process repeated. The isolated polysaccharide (16.0 g) had a ratio of rhamnose, fucose, xylose, mannose, galactose, and glucose of 5:9:23:21:13:28 and was inositol-free.

It was submitted to the Fehling solution procedures described above to provide a D-glucan and heteropolysaccharide.

Sugar composition of polysaccharide. — Polysaccharide was hydrolyzed with 0.5M H_2SO_4 for 18 h at 100°, the solution was made neutral ($BaCO_3$) and filtered, and the filtrate evaporated. The residue was converted into polyol peracetates by successive $NaBH_4$ reduction and acetylation with acetic anhydride–pyridine at 100°. Samples were analyzed in a g.l.c. column (120 × 0.4 cm i.d.) of 3% (w/w) ECNSS-M on Chromosorb W (80–100 mesh), from 130 to 180° (4°/min, then hold)[21]. In the case of heteropolysaccharide obtained *via* aqueous extraction, the sugar ratio was rhamnose, fucose, xylose, mannose, galactose, and glucose (4:10:32:29:17:8). In a different experiment where 2M trifluoroacetic acid at 100° for 12 h was used in the hydrolysis step, the ratio was 8:11:24:29:10:12.

Determination of the enantiomeric form of aldose components of the heteropolysaccharide. — After hydrolysis of the polysaccharide, fucose and galactose were isolated from the mixture by t.l.c. on silica gel G-60 using as solvent 200:47:15:1 (v/v) benzene–ethanol–water–acetic acid, and they were shown to have the L and D forms, respectively, by oxidation by L-fucose dehydrogenase[22] and D-galactose dehydrogenase[23]. Glucose was found to be oxidized by D-glucose dehydrogenase[24].

A hydrolyzate obtained from the heteropolysaccharide (25 mg) was fractionated by cellulose column chromatography, 19:1 (v/v) butanol–water eluting mixtures of rhamnose and fucose, and of fucose and xylose. Elution with 8:1 (v/v) butanol–water gave fractions containing xylose and mannose, and glucose. Finally, 4:1 (v/v) butanol–water eluted galactose. Each fraction was treated with (−)-2-octanol containing a trace of trifluoroacetic acid, at 130°, and converted to the octyl glycosides. These were acetylated and the resulting acetates examined by g.l.c.[8] in a quartz capillary column (0.32 mm i.d. × 60 m) containing chemically-bonded Durowax-4 (J. & W. Scientific). (Previously used[8] was SP-1000, but this was unavailable). Injections were made in the split mode at 50°, and a rapid program (40°/min) to 230° (hold) was carried out. The carrier gas was H_2 (linear velocity, 80 cm/s). The retention times of peaks corresponded to those of L-rhamnose, L-fucose, D-xylose, D-glucose, and D-galactose derivatives.

Methylation analysis of polysaccharides. — The samples were methylated and the products converted into partially *O*-methylated alditol acetates as previously described[10]. G.l.c.–m.s. was performed with a Model 4000 Finnigan unit, interfaced

with an Incos 2300 Data System. This was equipped with capillary columns (0.25 mm i.d. × 30 cm) coated with OV-17, DB-210, and 3:1 or 1:3 OV-225–OV-17. Electron-impact mass spectra were obtained repetitively every 2 s by scanning from mass 40 to 420. Injections were made in the split mode at 50° and a rapid program (40°/min) to 220° (hold) was carried out. The carrier gas was He (linear velocity, 22 cm/s). Final identification of products was obtained by co-injection with standards.

In the DB-210 column, the acetates of 6-*O*-methyl derivatives had different retention times depending whether they were derivatives of mannitol (8.12 min), glucitol (8.96 min), or galactitol (8.26 min). In the case of 2,6-derivatives, the values were: mannitol (7.08 min), glucitol (7.34 min), and galactitol (7.16 min). For the 3,4-di-*O*-methyl series, only the derivatives of mannitol (8.76 min) and galactitol (9.12 min) were tested.

Mixtures of *O*-methylalditol acetates, obtained from heteropolysaccharide and material derived from the Smith degradation, were examined in a capillary column of OV-17 under conditions previously described[9]. The polysaccharide product gave rise to, in the order of their emergence, acetates of 2,4,6-tri-*O*-methylgalactitol, 2,3,6-tri-*O*-methylglucitol, and 2,4,6-tri-*O*-mannitol and -glucitol in a ratio of 15:5:63:18. The ratio in the case of the Smith degradation product was 20:9:69:16.

On methylation of the heteropolysaccharide, 2,3-di-*O*-methylxylitol acetate, identified as a product by use of the 3:1 OV-225–OV-17 column, gave rise to an ambiguity since this compound has the same elution time as the 3,4-di-*O*-methyl isomer. To distinguish between the two compounds, the mixture of free *O*-methyl sugars was treated with 3% methanolic HCl at reflux for 3 h, and the product examined by g.l.c. using the DB-210 column, at a temperature of 50° programmed rapidly to 200° (hold). The mixture of methyl glycosides contained a peak corresponding to the methyl 3,4-di-*O*-methyl- (retention time 4.9 min), but not the methyl 2,3-di-*O*-methyl-xyloside (retention time 5.3 min).

ACKNOWLEDGMENTS

The authors thank Miss M. Baron and Mr. P. Odebrecht for help in collecting *C. pavonia* and the late Prof. R. J. G. Hertel of the Departamento de Botânica, Universidade Federal do Paraná, Curitiba, Brazil, for identification of the lichen. Thanks are due to Mr. Mazurek for recording the ^{13}C-n.m.r. spectra at the Plant Biotechnology Institute, N.R.C. of Canada, Saskatoon, Saskatchewan Canada. Amino acid analyses were kindly performed by Prof. F. S. Sosulski, Dept. of Crop Science, University of Saskatchewan, Saskatoon.

REFERENCES

1 P. A. J. GORIN AND M. IACOMINI, *Carbohydr. Res.*, 142 (1985) 253–267.
2 H. SAITÔ, T. OHKI, N. TAKASUKA, AND T. SASAKI, *Carbohydr. Res.*, 58 (1977) 293–305.
3 L. D. HALL AND F. JOHNSON, *J. Chem. Soc., Chem. Commun.*, (1969) 509–510.

4 J. O. PREVIATO, P. A. J. GORIN, R. H. HASKINS, AND L. R. TRAVASSOS, *Exp. Mycol.*, 3 (1979) 92–105.
5 P. A. J. GORIN, E. M. GIBLIN, G. P. SLATER, AND L. HOGGE, *Carbohydr. Res.*, 106 (1982) 235–238.
6 E. BARRETO-BERGTER AND P. A. J. GORIN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 67–103.
7 T. SASAKI AND N. TAKASUKA, *Carbohydr. Res.*, 47 (1976) 99–104.
8 K. LEONTEIN, B. LINDBERG, AND J. LÖNNGREN, *Carbohydr. Res.*, 62 (1978) 359–362.
9 E. BARRETO-BERGTER, L. HOGGE, AND P. A. J. GORIN, *Carbohydr. Res.*, 97 (1981) 147–150.
10 P. A. J. GORIN AND M. IACOMINI, *Carbohydr. Res.*, 128 (1984) 119–132.
11 B. LINDBERG AND B. WICKBERG, *Acta Chem. Scand.*, 7 (1953) 140–142.
12 B. LINDBERG, *Acta Chem. Scand.*, 9 (1955) 917–919.
13 A. D. ELBEIN, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 227–256.
14 G. TANRET, *C.R. Séances Hebd. Acad. Sci.*, 192 (1931) 1056–1058.
15 K. MYRBÄCK AND B. ÖRTENBLAND, *Biochem. Z.*, 288 (1936) 329–337.
16 T. S. HARDING, *Sugar*, 25 (1923) 476–477.
17 C. S. HUDSON, *J. Ind. Eng. Chem.*, 10 (1918) 176–178.
18 K. PAECH AND M. V. TRACEY, *Modern Methods of Plant Analysis*, Vol. II, Springer-Verlag, Berlin, 1955.
19 L. P. RIBEIRO, E. MITIDIERI, AND O. R. OFFONSO, *Paper Electrophoresis, a Review of Methods and Results*, Elsevier, Amsterdam, 1961, pp. 210–215.
20 S. F. DE ST GROTH, R. G. WEBSTER, AND A. DATYNER, *Biochim. Biophys. Acta*, 71 (1963) 377–391.
21 J. S. SAWARDEKER, J. H. SLONEKER, AND A. JEANES, *Anal. Chem.*, 43 (1965) 1602–1604.
22 M. F. GUIMARÃES, L. U. RIGO, AND L. A. VEIGA, in A. STOFANI, M. BACILA, AND B. L. HORECKER (Eds.), *Biochemistry and Genetics of Yeasts*, Academic Press, New York, 1978, pp. 161–170.
23 D. AMARAL, F. F. KELLY, AND B. L. HORECKER, *Methods Enzymol.*, 9 (1966) 87–92.
24 A. DAHLQVIST, *Biochem. J.*, 80 (1961) 547–555.