Structural Studies on a Galactomannan Isolated from the Lichen *Stereocaulon ramulosum*

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*Stereocaulon ramulosum* (SW.) Räusch, a lichen that grows in Southern Brazil, was treated with hot aqueous alkali, the extract fractionated with Fehling solution, and the resulting insoluble copper complex regenerated. The polysaccharide was fractionated stepwise in the presence of Cetavlon, and at pH 8.5, in the presence of borate, the predominant component was precipitated. From this, galactomannan B was obtained and shown to contain a main chain of (1→6)-linked α-D-mannopyranosyl units, which were unsubstituted (1), monosubstituted with single-unit side-chains of β-D-Galp-(1→4)- (2), and disubstituted with β-D-Galp-(1→4)- and α-D-Manp-(1→2)- (3). These principal components were arranged sequentially, with the structure 3→2 making a large contribution.

In lichens, the best-known polysaccharides are the linear isolichenans (α-glucans) and lichenans (β-glucans), and the branched-chain galactomannans. The chemical structures of these mycobiont components have been summarized in a recent review\(^1\) and often showed considerable variation from lichen to lichen. The galactomannans occur in all ascomycetous lichens and appear to arise from the cell wall, much like the mannose-containing polysaccharides of yeasts.\(^2\) The former have a main chain of (1→6)-linked α-D-mannopyranosyl units with side chains of different structures with the possibility of β-D-Galp-(1→4), α-D-Galp-(1→2) and α-D-Manp-(1→2) units arranged as monosubstituents or as disubstituents on the same main-chain residue. Our objective was to determine the chemical structures of different lichen galactomannans, and to use parent lichen or cell walls to provoke antibodies, which could be isolated using columns of immobilized galactomannan. Such specific antibodies could be utilized in the identification of similar structures in glyco-complexes or in polysaccharides that are available in only minute quantities, as is the case with certain protozoa.\(^3\) The correlation of proton NMR signals with those of galactomannan or derived disaccharides could also be used in structural identification. It has also been found, in the case of yeasts, that the structure of NMR spectra of mannose-containing polysaccharides could be used in chemotaxonomy,\(^2\) an approach that might be similarly applied to lichens.

*Stereocaulon ramulosum* has been found to contain the carbohydrates umbilicin, α-α-D-trehalose, an α-D-glucan resembling isolichenan and a linear (1→3)-linked β-D-glucopyranan, which had a structure not previously found in lichens.\(^4\) In this investigation, the α-D-glucan was isolated via hot aqueous extraction followed by Fehling precipitation and recovery from the supernatant. The insoluble copper complex which formed was mainly derived from a galactomannan, which was not further investigated, but is now the subject of a continuation of our studies on lichen galactomannans.

**Materials and Methods**

*General analytical methods.* Specific rotations were obtained using a Perkin Elmer model 141 automatic polar-
mometer on solutions of about 0.5% concentration. Total carbohydrate was determined using the phenol-sulfuric acid method, and protein with the Folin-Ciocalteau reagent. The periodate consumed and formic acid produced, using galactomannan as the substrate, were estimated using 0.05 m NaIO₄ in the dark at 0~2°C, the values leveling off after 72 hr.

Isolation of galactomannan A following hot aqueous extraction. St. ramulosum (50 g) was successively extracted with 9:1 benzene-ethanol (v/v) and 80% aqueous methanol, and the residue divided into two equal portions. One of them (20.1 g) was extracted with water (500 ml) at 100°C for 8 hr. The combined filtrates were concentrated to 75 ml, added to excess ethanol, and the resulting precipitate (5.2 g) isolated after centrifugation. This precipitate was solubilized in hot water (100 ml), the solution frozen, and then thawed at 4°C. The precipitate which formed was centrifuged off, and the freezing and thawing process repeated on the supernatant until no more precipitate appeared. The supernatant was then treated with Feuling solution (50 ml), and the precipitated copper complex isolated and converted to a polysaccharide. This was dissolved in water (15 ml) and fractionally precipitated in the presence of 5% Cetavlon in water (15 ml). The precipitate, which formed at pH 7.0, was dissociated with 4 m NaCl, and upon its addition to excess ethanol, a polysaccharide was precipitated and isolated in a 13 mg yield. The supernatant of the Cetavlon precipitate was treated with 3% aqueous sodium tetraborate (30 ml), which resulted in a solution of pH 8.5, giving a precipitate which was isolated, the borate complex being decomposed with acetic acid and added to excess ethanol, which precipitated galactomannan A (0.12 g). The supernatant was adjusted to pH 12.0 with aqueous sodium hydroxide and, in the presence of 3 volumes of ethanol, a precipitated complex appeared and was converted to a glucan (4 mg).

The supernatant of the Fehling precipitation was neutralized with acetic acid, dialyzed against water, and deionized. This solution was evaporated to a small volume, and the component glucan (0.98 g) precipitated with excess ethanol.

Isolation of galactomannan B via a hot aqueous alkaline extraction of lichen. The other half of the lichen sample (20.1 g), after extracting with organic solvents, was treated with 2% aqueous KOH (500 ml) at 100°C for 2.2 hr. The mixture was then filtered, neutralized with acetic acid, and concentrated to a small volume (140 ml). This concentrate was added to excess ethanol, and centrifugation of the precipitate led to the isolation of a polysaccharide (8.2 g), which was submitted to the Fehling and Cetavlon procedures already described. The following polysaccharides were obtained: pH 7.0 (29 mg), and pH 8.5 in the presence of borate (galactomannan B, 0.87 g); pH 12.0 in the presence of borate (glucan, 22 mg). α-D-Glucan (0.76 g) was isolated from the supernatant of the Fehling pre-cipitation.

Isolation of polysaccharide via DMSO extraction of the lichen. Lichen (5.0 g), which had been previously been extracted with benzene-ethanol, was stirred in DMSO (100 ml) at room temperature for 89 hr. The mixture was filtered through cotton wool, and the filtrate then centrifuged to remove the fine material. The supernatant was added to excess ethanol, and the precipitated polysaccharide (0.26 g) isolated by filtration.

Partial acid hydrolysis of galactomannan B. A solution of galactomannan (0.20 g) in 0.16 m H₂SO₄ (20 ml) was maintained at 100°C for 18 hr, neutralized (BaCO₃), filtered, and the filtrate evaporated to a small volume. Addition to excess ethanol precipitated a polysaccharide (31 mg), which was isolated by centrifugation.

Partial acetolysis of galactomannan B. Galactomannan (243 mg) was shaken for 5 days in acetic anhydride–AcOH–H₂SO₄ and the product isolated and de-O-acetylated. The resulting mixture of mono- and oligosaccharides was fractionated from a column of cellulose with successive acetone–water mixtures of 9:1, 4:1, 3:1 and 2:1 (v/v). Obtained were mannose, galactose, and oligosaccharide fractions A (16 mg) and B (24 mg).

Gas-liquid chromatography, with and without mass spectrometry. GLC-MS was used to identify the substitution positions in partly O-methylated alditol acetates formed from the methylation analysis of galactomannans A and B. A sample of each was successively methylated by the methods of Haworth and Kuhn et al., and the per-O-methylated product hydrolyzed with cold 72% H₂SO₄ and then by hot dilute H₂SO₄ to form a mixture of partly O-methylated aldoses. This mixture was reduced with aqueous NaBH₄, and the product acetylated to give partly O-methylated alditol acetates, which were submitted to GLC-MS using a Model 4000 Finnegan unit interfaced with an INCOSS data system. Electron impact spectra were obtained repetitively every 2 sec, scanning from m/z 40 to 420. A capillary column (0.25 mm i.d. x 30 m) of a 3:1 blend of OV-225 and OV-17 was used. Injections were made in the split mode at 50°C, and a rapid program at 40°C/min to 220°C (then hold) was carried out. The carrier gas was He with a linear velocity of 22 cm/sec. Identification of the resulting peaks was achieved by electron impact (ionization) MS breakdown patterns and retention times.

The monosaccharide compositions of the oligo- and polysaccharides were found by GLC quantitation of the aldito acetates formed by treatment with 2 m TFA at 100°C for 8 hr, with subsequent borohydride reduction and acetylation. A Varian model 2440 chromatograph was used, having a conventional column of 3% OV-225 on 100~200 mesh Gas Chrom Q (0.15 i.d. x 200 cm) at 190°C with N₂ as the carrier gas. The same column and a
temperature of 170°C were employed in order to identify methyl 2,4,6-tri-O-acetyl-3-O-methyl-mannopyranoside, rather than the 4-O-methyl isomer, in the mixture of partly O-methylated aldoses (see above) by treating with refluxing methanolic HCl and subsequent acetylation.16)

Carbon-13 and proton nuclear magnetic resonance spectroscopy. NMR spectra were obtained using a Bruker AM-360 WB spectrometer incorporating a Fourier transform. Samples were dissolved in D_{2}O and run at 33°C (H-NMR) and 70°C (13C-NMR). Chemical shifts are expressed as δ in ppm relative to the resonance of Me_{4}Si (TMS, δ=0), which was determined in separate experiments.

Paper chromatography. This was carried out on Whatman no. 1 filter paper, using benzene-1-butanol-pyridine-H_{2}O (1 : 5 : 5 : 3, v/v upper layer) as a general solvent, and 1-butanol-ethanol-water 40 : 1 1 : 1 19, v/v) for detecting the Smith degradation products of galactomannan B and glucan. Alkaline AgNO_{3} and β-anisidine hydrochloride were employed as spray reagents. D-Galactose found in the acid hydrolyate of galactomannan B was characterized by the D-galactose oxidase method.17)

Characterization of α-D-glucan. The glucan was identified by using the techniques outlined in a previous publication.40

Smith degradations of α-D-glucan and galactomannan B. Each polysaccharide was submitted to Smith degradations, one series incorporating mild and the other strong acid hydrolysis conditions.4) The following fragments were identified.

Under mild hydrolytic conditions, the α-D-glucan gave spots, corresponding on a paper chromatogram to erythritol, 2-O-α-D-glucopyranosyl-D-erythritol and O-α-D-glucopyranosyl-(1→3)-O-α-D-glucopyranosyl-(1→2)-D-erythritol, which were obtained in a similar degradation of isolichenan.18) Galactomannan B gave rise to strong spots on a paper chromatogram of glycerol and to an unknown moving slightly more slowly than mannose, along with a weaker one of erythritol. The unknown was isolated by preparative chromatography on plates of silica-gel G (ethyl acetate-methanol-H_{2}O solvent, 7 : 3 : 2, v/v) and was hydrolyzed to give mannitol and erythritol.

Using strong hydrolytic conditions in the Smith degradations, paper chromatography showed that the α-D-glucan gave rise to glucose and erythritol and galactomannan B to mannose, erythritol and glycerol.

Results and Discussion

*St. ramulosum* was successively extracted with benzene-ethanol and 80% aqueous methanol in order to remove the low molecular weight material, and the residue was then extracted with hot water, the liberated polysaccharides being fractionated. In another series of experiments, polysaccharide extraction was carried out with hot aqueous alkali, identical fractionation procedures being used. The present study concerns a detailed analysis of galactomannan liberated by the second procedure.

In a previous investigation,4) the hot aqueous extract of lichen was added to excess ethanol, and the precipitated polysaccharide (21% yield) was shown to contain glucose, galactose and mannose in a molar proportion of 18 : 1 : 1. When this was dissolved in hot water, which was then frozen and gradually thawed, a precipitate formed which contained galactose, mannose, glucose, xylose, fucose and rhamnose in a molar ratio of 48 : 12 : 26 : 4 : 5 : 5. As it was a minor component, this precipitate was not examined further and attention was only paid to an α-D-glucan (4.5% yield), which remained in the supernatant after a Fehling precipitation step, and which proved to be structurally similar to isolichenan.

We have now isolated the insoluble copper complex and converted it to a polysaccharide containing galactose, mannose, fucose, rhamnose and xylose, which were present in a molar proportion of 69 : 22 : 3 : 3 : 4, along with a uronic acid. As these components suggested a galactomannan contaminated with other polysaccharides, the material was submitted to a further fractionation using aqueous Cetavlon. At pH 7.0, an insoluble complex was formed with acidic heteropolysaccharide, which was recovered (0.05% yield) and found to contain galactose, mannose, glucose, fucose, xylose and rhamnose in a molar ratio of 45 : 14 : 4 : 11 : 23 : 4, as well as uronic acid. The high proportion of xylose was unexpected. Since the specific rotation was low at −76°, negative contributions could have been made by α-L-fucopyranosyl, α-L-rhamnopyranosyl and β-D-xlyopyranosyl units. Levorotatory β-D-galactofuranosyl residues were not present, since typical C-1 signals at δ ≥ 106.6 in the 13C-NMR spectrum19) were not observed. After
adjusting the Cetavlon system to pH 8.5 with aqueous sodium tetraborate, the major component galactomannan A (0.49% yield; 5.9% protein) was precipitated and found to contain only galactose and mannose in a 3:2 molar ratio with a specific rotation of +52°. The supernatant was then treated with aqueous sodium hydroxide, giving a pH of 12.0, and its addition to ethanol resulted in the precipitation of a complex of glucan, which was isolated in only a 0.02% yield.

As the yield of galactomannan was low, the lichen was extracted more exhaustively with hot aqueous KOH, giving polysaccharide in a 33% yield. A higher proportion of galactomannan was obtained, by virtue of the presence of galactose, mannose, glucose, xylose and rhamnose in a molar ratio of 28:24:47:0.3:1. The process of dissolution in water, followed by freezing and thawing, gave rise to an insoluble mixture, which should have contained a previously identified (1→3)-linked β-D-glucopyranan, as well as a heteropolysaccharide. Although these polysaccharides could be of interest as components of the phycobiont of the lichen, their yield was small and the fraction was not further examined. The supernatant that was obtained after thawing was treated with Fehling solution, resulting in the formation of an insoluble Cu complex. Fractionation of its component polysaccharides with Cetavlon provided heteropolysaccharide (pH 7.0; 0.12% yield, also a phycobiont component?), galactomannan B (pH 8.5 with borate; 3.5% yield, 3% protein) and glucan (pH 12.0 with borate; 0.09% yield). α-D-Glucan was recovered from the supernatant of the Fehling precipitation in a 3.1% yield.

In order to test for O-acetyl groups in component glucans, the lichen was extracted with cold DMSO, as in a previous study when a lightly O-acetylated (1→6)-linked β-D-glucopyranan was extracted from a lichen and O-acetate esters were identified by a typical 13C-NMR signal at δ about 22. However, although the extraction was efficient (5.2% yield), with both glucan and galactomannan components being obtained (the molar ratio of galactose, mannose, glucose, arabinose, xylose and fucose was 26:12:55:3:1:3), O-acetyl groups were not detected. It is noteworthy that in the DMSO extraction of some lichens, glucans were sometimes obtained pure, and in others were contaminated with galactomannans. This appears to have been due to the accessibility of the latter, as controlled by its linkage to the lichen cell-wall. In the case of St. ramulosum, galactomannan was isolated as linked to protein, but since glucosamine was not detected by paper chromatography following strong acid hydrolysis, the linkage of galactomannan to the cell wall did not involve a glucosamine-asparagine bridge, as in the case of certain ascomycetes such as baker’s yeast.

As galactomannan B was isolated in a higher yield than galactomannan A, it is more representative of the overall galactomannan composition of the lichen and was preferred as the subject of a detailed chemical analysis. It contained galactose and mannose in a molar ratio of 46:54, had a specific rotation of + 49°, and a methylation analysis gave rise to a number of partly O-methylated alditol acetates, whose structures (Table I) correspond to the non-reducing end-units of galactopyranose (41%); mannopyranose (14%), 2-O (1%) and 6-O-substituted mannopyranosyl residues (8%), and others that were 4,6-di-O (17%) and 2,6-di-O-substituted (2%). Present also were 3,4,6-tri-O (2%), 2,4,6 or 2,3,6-tri-O-substituted mannopyranosyl units (11%), this ambiguity arising since 3-O and 4-O-methylmannitol are identical. This was resolved by treating the mixture of partly O-methylated alditol acetates, whose structures (Table I) correspond to the non-reducing end-units of galactopyranose (41%); mannopyranose (14%), 2-O (1%) and 6-O-substituted mannopyranosyl residues (8%), and others that were 4,6-di-O (17%) and 2,6-di-O-substituted (2%). Present also were 3,4,6-tri-O (2%), 2,4,6 or 2,3,6-tri-O-substituted mannopyranosyl units (11%), this ambiguity arising since 3-O and 4-O-methylmannitol are identical. This was resolved by treating the mixture of partly O-methylated alditol acetates, whose structures (Table I) correspond to the non-reducing end-units of galactopyranose (41%); mannopyranose (14%), 2-O (1%) and 6-O-substituted mannopyranosyl residues (8%), and others that were 4,6-di-O (17%) and 2,6-di-O-substituted (2%). Present also were 3,4,6-tri-O (2%), 2,4,6 or 2,3,6-tri-O-substituted mannopyranosyl units (11%), this ambiguity arising since 3-O and 4-O-methylmannitol are identical. 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Table I. GLC Analysis of Partially O-Methylated Alditol Acetates Obtained from Methylated Carbohydrates

<table>
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<tr>
<th>O-Methylalditols</th>
<th>Galactomannan A</th>
<th>Galactomannan B</th>
<th>Acid-degraded galactomannan B</th>
<th>Fractions obtained via partial acetylation of galactomannan B</th>
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<tr>
<td>Hex</td>
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</table>

a Proportion (%) of the peak area relative to the total peak areas.
b Analyzed as peracetates.
c Capillary column of 3:1 OV-225-OV-17.
d Conventional column of 3% OV-225.

dation, incorporating strong hydrolytic conditions, gave a mixture of mannose, erythritol and glycerol, which on borohydride reduction and subsequent acetylation gave rise to acetates of mannitol, erythritol and glycerol in a molar ratio of 20:11:69 (GLC). This indicates that 20% of the mannopyranosyl main-chain units were substituted at O-2,4,6, and 11% at O-4,6.

Partial acid hydrolysis of galactomannan B removed some of the side chains, furnishing the core in a 16% yield. This contained mannose and galactose in a molar ratio of 84:16, and a methylation analysis (Table I) showed end groups of mannopyranose (15%) and galactopyranose (12%), with 4,6-di-O (6%) and 2,6-di-O-substituted mannopyranosyl units (11%). The main structure was 6-O-substituted mannopyranosyl residues (54%), which are likely components of a main chain having this structure entirely. The specific rotation of the polysaccharide was +60°, consistent with that of α-D-mannopyranosyl units.8) The acid hydrolyzate of galactomannan B contained D-galactose, since it was oxidized by D-galactose oxidase.17) Since the specific rotation of the galactomannan is +49°, the galactopyranosyl units had a weak rotational contribution and should have mainly had the β-D-configuration.

Partial acetylation of galactomannan B and subsequent de-O-acetylation provided a mixture of mannose, galactose, and di- and trisaccharide, according to paper chromatography. Fractionation on a column of cellulose gave monosaccharide fractions, fraction A (6% yield), whose major component had an Rf corresponding to 2-O-a-D-mannopyranosyl-D-mannose, along with a trace of a slightly slower moving material co-chromatographing with 6-O-a-D-mannopyranosyl-D-mannose, and fraction B (9% yield), which gave rise to the same disaccharide spots as well as to a small one with the Rf of a (1→2)-linked mannotriose.

Fraction A proved to be complex, since hydrolysis provided galactose (23%) and mannose (72%), and the borohydride-reduced material, galactose, mannose and hexitol. A
methylation analysis, which incorporated the Kuhn technique (Table I), showed non-reducing end-groups of mannopyranose (21%) and galactopyranose (23%), with 2-O (22%), 4-O (20%) and 6-O-substituted (14%) reducing end-groups of mannose. These data can be rationalized in the light of the $^{13}$C-NMR spectrum of fraction A (Fig. 1A), which is consistent with a mixture of 2-O-$\alpha$-D-mannopyranosyl-D-mannose, with signals at $\delta$ 103.8 (C-1'), 94.2 (C-1) and 80.7 (C-2), and 4-O-$\beta$-d-galactopyranosyl-D-mannose. This structure is indicated by signals typical of the non-reducing end-units of galactopyranose at $\delta$ 104.7 (C-1') and 77.0 (C-5'), and by that of 4-O-substituted mannopyranose at $\delta$ 78.3 (C-4). The $^1$H-NMR spectrum of fraction A (Fig. 1C) contained signals of approximately equal magnitude at $\delta$ 5.30 and 4.96, arising from H-1' and H-1 of 2-O-$\alpha$-D-mannopyranosyl-D-mannose, along with a minor one at $\delta$ 4.36, whose large coupling constant of 7.7 Hz is typical of the $\beta$-configuration in galactopyranose. A signal at $\delta$ 5.10 was of similar size, suggesting that it arose from the reducing end of the same disaccharide. The H-1 signal at $\delta$ 4.83 corresponds to those of 6-O-$\alpha$-D-mannopyranosyl-D-mannose.

Fraction B was a complex mixture, but with 4-O-$\beta$-D-galactopyranosyl-D-mannose in a higher proportion, which is reflected in the molar ratio of galactose to mannose of 41:59. Although the methylation data is inaccurate, due to concomitant oxidation with silver oxide at the reducing ends of the disaccharides, that resulted in lower than expected amounts of tri-O-methylmannitol acetates, it is still possible to identify the fragments qualitatively. These results combined with the quantitative data (Table I) showed end groups of galactopyranose (46%) and mannopyranose (18%), and reducing groups of mannose substituted at O-2 (7%), O-4 (19%) and O-6 (11%). Fraction B gave a $^1$H-NMR spectrum (Fig. 1D)
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with identifiable H-1 signals at δ 4.83 (6-O-α-D-mannopyranosyl-D-mannose), and large ones at δ 5.10, J = 1.6 Hz (H-1) and δ 4.36, J = 7.7 Hz (H-1') of 4-O-β-D-galactopyranosyl-D-mannose. This is the first time that this disaccharide has been characterized in the partial acetolysate of a lichen galactomannan, more common ones having been 2-O-α-D-mannopyranosyl-D-mannose and 2-O-α-D-galactopyranosyl-D-mannose.¹⁶

The overall evidence indicates that galactomannan B has a (1→6)-linked α-D-mannopyranosyl main-chain, which is unsubstituted (about 8%, 1), according to methylation data. Employing the methylation data qualitatively

\[
\begin{align*}
\beta-D-Galp & \\
1 & \\
\downarrow & \\
-\alpha-D-Manp-(1\to6)- & -\alpha-D-Manp-(1\to6)-
\end{align*}
\]

\[
\begin{align*}
\beta-D-Galp & \\
1 & \\
\downarrow & \\
-\alpha-D-Manp-(1\to6)- & -\alpha-D-Manp-(1\to6)- & -\alpha-D-Manp-(1\to6)- & -\alpha-D-Manp-(1\to6)-
\end{align*}
\]

\[
\begin{align*}
\alpha-D-Manp
\end{align*}
\]

and basing quantitation on the Smith degradation under strong hydrolytic conditions, where acetates of glycerol, erythritol and mannitol were examined by GLC, the erythritol proportion shows that 11% of the main-chain units are substituted at O-4 by β-D-Galp units (2), whereas that of mannitol is consistent with 20% of the main-chain units being disubstituted at O-2 and O-4 with α-D-Manp and β-D-Galp, respectively (3). The structural components 1, 2 and 3 thus comprise about 90% of the galactomannan.

Evidence for the sequence of the three structures was obtained by a Smith degradation under mild hydrolytic conditions of galactomannan B. Examination of the product on paper chromatograms showed spots of glycerol and a component moving slightly more slowly than mannose, along with a small one of erythritol. The unknown component was isolated by preparative silica-gel chromatography, giving mannose and erythritol on hydrolysis, and should have arisen from 1-O-α-D-mannopyranosyl-L-erythritol. This is consistent with a predominant 3→2 sequence in the polysaccharide, leaving 1→2 and/or 2→2 sequences that would provide the small proportion of erythritol.

Both the ¹³C-NMR spectra of galactomannan A (Fig. 1B) and B contained a large C-1 signal at δ 104.7, arising from β-D-Galp units.¹⁹ The only difference in the spectra is that the former contained small signals of β-Galp units, including that of C-1 at δ 109.4. This is consistent with the methylation data (Table I), which indicates 2% of non-reducing end-units of Galp. As this structure is not found in galactomannan B (according to methylation data too), it appears to have been present in a glycocomplex, which was decomposed by the action of alkali, and the carbohydrate component did not appear in the ethanol precipitate.

The glucan obtained via alkaline extraction was less pure that that obtained following aqueous extraction, as it contained 9% each of galactose and mannose. However, its structure was suggested to be the same, as its ¹³C-NMR spectrum was also similar to that of isolichenan.¹⁶ Controlled Smith degradation also gave erythritol, 2-O-α-D-glucopyranosyl-D-erythritol and O-α-D-glucopyranosyl-(1→3)-O-α-D-glucopyranosyl-(1→2)-D-erythritol, which were detected on paper chromatograms. The specific rotation of the glucan was +151°, the presence of impurities decreasing that of +171°, which was found for pure α-D-glucan.⁴ A negative iodine test showed that amyllose was absent, although such an impurity was present in related glucans prepared from Cetraria islandica²⁸ and Ramalina usnea.¹⁶

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References


