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POLYSACCHARIDES OF THE LICHENS *Cetraria islandica* AND *Ramalina usnea**

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ABSTRACT

Cetraria islandica (Iceland moss) and *Ramalina usnea,* lichens having a fruticose-growth form and ascomycetous mycosymbionts, contain D-galacto-D-mannans with structures differing from any previously recognized. The polysaccharides are related, having (1→6)-linked α-D-mannopyranosyl main-chains. However, the *C. islandica* polysaccharide is more highly branched with α-D-Gal*p*-(1→2)- and β-D-Gal*p*-(1→4) side-chains linked to the same α-D-mannopyranosyl residues as in the preponderant structure {→6)-[α-D-Gal*p*-(1→2)]-[β-D-Gal*p*-(1→4)]-α-D-Man*p*-(1→6)-α-D-Man*p*-(1→}. The *R. usnea* D-galacto-D-mannan contains β-D-Gal*p*-(1→4)-linked groups. *R. usnea* does not contain a β-D-glucan-resembling lichenan but has an α-D-glucopyranan preponderant repeating structure with three consecutive (1→3) links interspersed with single (1→4) links.

INTRODUCTION

A polysaccharide was recognized as a component of a lichen as early as 1815 when Berzelius[1] isolated a material from *Cetraria islandica* (Iceland moss) following its precipitation on cooling a hot-water extract. Lichenan, thus isolated, can be further purified *via* its insoluble copper complex formed with Fehling solution[2]. It is a β-D-glucopyranan[2,3] containing (1→3) and (1→4) linkages in a molar ratio of 3:7. The polysaccharide mixture obtained from the supernatant solution on cooling of the hot-water extract just mentioned was originally called isolichenan by Meyer and Gürtler[4]. However, Chanda *et al.*[2] later applied this name to the α-D-glucopyranan component that was purified by fractional precipitation, with acetone, of the water-soluble copper complex. They found (1→3)- and (1→4)-linkages in a molar ratio of 3:2, although Peat *et al.*[5] later suggested a ratio of 11:9. The fractionation procedure of Chanda *et al.*[2] presumably separated lichenan from a component containing D-mannose and D-galactose which was detected as early as 1906 by Ulander and Tollens[6]. Buston and Chambers[7] suggested the presence of a

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hemicellulose by virtue of the presence of galacturonic acid in addition to D-galactose and D-mannose. However, the presence of the uronic acid has not since been confirmed.

Karrer and Joos[8] found that, following removal of lichenan by cold-water precipitation, treatment of the supernatant solution with Fehling solution resulted in the formation of an insoluble copper complex containing mannose, galactose, and glucose in a molar ratio of 21:35:44. Such an observation suggests the co-precipitation, as copper complexes, of a galactomannan with remaining lichenan. However, a heteropolymeric component of *C. islandica* was not isolated in a pure state. The only finding on its possible structure was provided by Granichstädten and Percival[9] who, on treatment of an aqueous sodium hydroxide extract with Fehling solution, obtained a precipitate consisting mainly of D-glucan with a small proportion of heteropolysaccharide. This was found to contain nonreducing end groups of D-galactopyranose. The present detailed study shows the heteropolymer to be a D-galacto-D-mannan, whose structure was compared with that of *Ramalina usnea* (L.) R. H. Howe. The α-D-glucan of *R. usnea* was also investigated.

RESULTS AND DISCUSSION

Polysaccharide of Cetraria islandica. — *C. islandica* was extracted with hot water, and the lichenan, which formed on cooling, was removed in the usual way. The supernatant solution was evaporated, frozen, and following slow thawing at 4° further insoluble lichenan was removed. Treatment of the supernatant solution with Fehling solution gave an insoluble copper-complex which provided the polysaccharide in 0.7% yield. It contained mannose, galactose, and glucose in a molar ratio of 43:40:17. The preparation was methylated and the resulting *O*-methylated product converted into partially *O*-methylated alditol acetates. These were analyzed by g.l.c.–m.s. using a glass capillary column containing OV-17 and OV-225 in a 1:3 ratio[10]. The resulting fragments and their proportions are presented in Table I, which shows that the principal compounds present are nonreducing end groups of galactopyranose (36%) and mannopyranose (7%), and 6-*O*-mono- (19%) and 2,4,6-tri-*O*-substituted (15%) residues of mannopyranose. [Since the formation of 3-*O*-methylmannitol pentaacetate (≡ 4-*O*-methyl derivative) was equivocal, the methylated polysaccharide was converted into methyl *O*-methylhexoside acetates. G.l.c. analysis showed that only the 3-*O*-methyl derivative was present]. Minor proportions of *O*-methylalditol acetates derived from 2,3,4,6-tetra-*O*- (3%), 2,4,6- (4%), and 2,3,6-tri-*O*-methyl-glucitol (4%) were also found. Most of the tri-*O*-methyl derivatives apparently arose from lichenan, since treatment of the polysaccharide with a cellulase from *Aspergillus niger* gave a product (ratios of mannose to galactose to glucose of 49:45:6) which contained few 3-*O*- and 4-*O*-substituted glucopyranose residues (see methylation results in Table I). Diminution of the proportions of nonreducing end-groups of galactopyranose indicate that enzymic removal of some of these residues had taken place.

TABLE I

G.L.C. ANALYSIS OF PARTIALLY *O*-METHYLATED ALDITOL ACETATES OBTAINED FROM METHYLATED POLYSACCHARIDES

Alditol	T_M[a]	*Percentage of total peaks obtained from methylated polysaccharide*					
		C. islandica			R. usnea		
		D-*Galacto*-D-*mannan*			D-*Galacto*-D-*mannan*		α-D-*Glucan*
		From Fehling precipitate (A)	*Cellulase treated A (B)*	*Acid degraded B (C)*	*From precipitate (G)*	*Acid degraded G*	
2,3,4,6-Me_4-Man	1.00	7	2	13	2	11	
2,3,4,6-Me_4-Glc	1.01	3	1				4
2,3,5,6-Me_4-Gal	1.03	2	2		3		
2,3,4,6-Me_4-Gal	1.07	36	32	11	40	14	
3,4,6-Me_3-Man	1.28	2	1				
2,4,6-Me_3-Glc	1.30	4	1	2			69
2,3,6-Me_3-Man	1.33	1					
2,3,6-Me_3-Gal	1.35	3	3	1			
2,4,6-Me_3-Man	1.38		1				
2,3,4-Me_3-Man	1.41	19	29	57	19	66	
2,3,6-Me_3-Glc	1.43	4					22
2,6-Me_2-Glc	1.73						3
4,6-Me_2-Glc	1.77						2
2,3-Me_2-Man	1.88	4	6	7	37	7	
3,4-Me_2-Man	1.98	3	5	8		2	
3-Me-Man	2.76	15	17	1			

[a]Retention time (T_M) relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol.

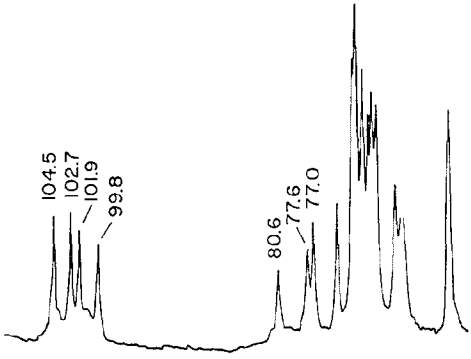


Fig. 1. ^{13}C-n.m.r. spectrum of *C. islandica* heteropolymer (Fraction A) in deuterium oxide at 70°. Numerical values are δ in p.p.m.

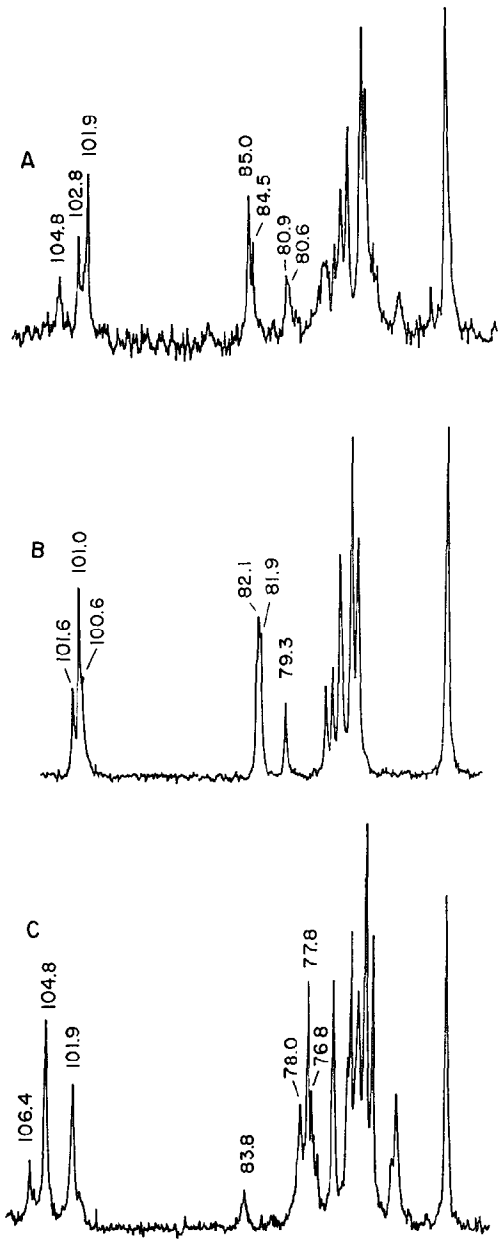
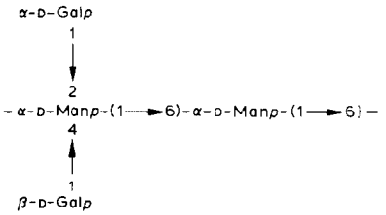


Fig. 2. ^{13}C-n.m.r. spectra of polysaccharides in deuterium oxide at 70° (numerical values in δ, p.p.m.): (A) Water-insoluble polysaccharide from *R. usnea*. (B) α-D-Glucopyranan from *R. usnea*. (C) D-Galacto-D-mannan from *R. usnea*.

In order to determine the structure of the galactomannan main-chain, the enzyme-treated material was subjected to partial hydrolysis with 0.16M sulfuric acid for 18 h at 100°, which preferentially removed galactosyl groups. The resulting ethanol-insoluble polysaccharide contained mannose, galactose, and glucose in the ratios of 74:21:5. Its ^{13}C-n.m.r. spectrum resembled that of a (1→6)-substituted α-D-mannopyranan structure since it had principal signals[11] at δ 101.0, 72.5 (2 signals), 71.6, 68.5, and 67.5. Methylation analysis (Table I) indicated the presence of 57% of the 6-*O*-substituted mannopyranosyl residues, 11% of nonreducing end-groups of galactopyranose and 13% of mannopyranose, and 7% of 4,6- and 8% of 2,6-di-*O*-substituted residues of mannopyranose. In view of the positive specific rotation (+51°) of the acid-degraded polysaccharide, which contains mainly α-D linkages according to the ^{13}C-n.m.r. spectrum, the mannosyl residues were attributed the D-configuration. The galactosyl residues also have this configuration since they were oxidized by D-galactose oxidase[12].

Thus, it appeared that the D-galacto-D-mannan contains a high proportion of (1→6)-linked α-D-mannopyranosyl units in the main chain. These are either unsubstituted or 2,4-di-*O*-substituted by D-galactopyranosyl groups. According to the ^{13}C-n.m.r. spectrum (Fig. 1), which showed a C-1 signal at the low field of δ 104.5, the D-galactopyranosyl groups, linked (1→4) to α-D-mannopyranosyl residues (see later ^{13}C-n.m.r. data of *R. usnea* D-galacto-D-mannan; Fig. 2c) have the β-D-configuration[13]. Also consistent with this interpretation is the presence of a typical[14] signal for C-5 at δ 77.0. The signal for C-1 at δ 102.7 suggested β-D-galactopyranosyl groups substituting O-2 of a (1→6)-linked α-D-mannopyranosyl main-chain, as in the D-galacto-D-mannan of *Saccharomyces octosporus*[14]. This structure was confirmed by isolation of 2-*O*-α-D-galactopyranosyl-D-mannose, following partial acetolysis of the *C. islandica* polysaccharide. The ^{13}C-n.m.r. spectrum of the D-galacto-D-mannan showed C-1 signals typical[15] of α-D-mannopyranosyl residues that are 6-*O*- (δ 101.9) and 2,4,6-tri-*O*-substituted (δ 99.8). The latter have a resonance close[15] to that of 2,6-di-*O*-substituted residues. Also present is a C-2 signal for 2-*O*-substituted D-mannopyranosyl residues (δ 80.6).

Smith degradation with mild hydrolytic conditions of the galactomannan gave 1-*O*-α-D-mannopyranosyl-L-glycerol, indicating a high proportion of alter-



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nate 6-*O*- and 2,4,6-tri-*O*-substituted α-D-mannopyranosyl residues, as shown in structure **1**. The fragment was characterized, following isolation by cellulose-column chromatography, by acid hydrolysis which yielded mannose and glycerol. The positive specific rotation of +55° of the mannopyranosylglycerol indicated an α-D configuration for the D-mannopyranosyl residue. Also the ^{13}C-n.m.r. spectrum showed nine signals with two of them, at δ 62.7 and 64.3, corresponding to two unsubstituted primary hydroxyl groups, thus confirming substitution at O-1 of the glycerol unit.

Lichen heteropolysaccharides have been isolated previously from *Cladonia alpestris* (reindeer moss) and *Evernia prunastri*. Aspinall *et al.*[16] obtained, from reindeer moss *via* precipitation with Fehling solution, products containing galactose, mannose, and glucose. Although the methylation data apparently differed from those obtained for the *C. islandica* preparations, the analytical techniques used in 1955 do not preclude similarities in chemical structure. Another heteropolymer from *E. prunastri* contains galactose, mannose, and glucuronic acid[17]. Its only possible similarities to structure **1** are the high proportions of non-reducing end-groups of galactopyranose (36%) and mannopyranose (11%), and tri-*O*-substituted mannopyranosyl residues, whose positions of substitution were not confirmed. In view of these uncertain results, heteropolysaccharides from another lichen having an ascomycete as mycosymbiont was investigated.

Polysaccharide of Ramalina usnea. — Following extraction of *Ramalina usnea* with 9:1 benzene–ethanol at reflux, the polysaccharide was extracted with hot 80% aqueous methanol. G.l.c.–m.s. analysis of acetates of soluble material showed the presence of derivatives of glycerol (6%), erythritol (1%), unknowns (12%), arabinitol (74%), and xylitol (8%). The arabinitol (3% yield based on lichen) was identified as the D enantiomer. The remaining insoluble lichen was extracted with hot water, and, on evaporation to a small volume followed by cooling, a small amount of glucan (0.5% yeld) precipitated. Methylation analysis showed it to contain (1→3) and (1→4) linkages in a ratio of 31:19, and its ^{13}C-n.m.r. spectra (Fig. 2A) showed mainly signals for C-1 at δ 102.8 and 101.9 typical of the preponderant α-D-glucopyranan component remaining in the supernatant solution (see Fig. 2B). A minor signal for C-1 at δ 104.8 corresponds to a signal of a D-galacto-D-mannan component (see Fig. 2C), rather than to that of lichenan, which also would give other signals at δ 105.1 and 106.0. Although the insoluble product gave, with iodine, a blue color corresponding to that of amylose, it was present in a very small proportion as the ^{13}C-n.m.r. spectrum did not contain a typical signal for C-1 at δ 101.3.

The supernatant solution of the precipitation from cold water just described was treated with excess ethanol, and the resulting precipitate (7% yield) found to contain α-D-glucan and a D-galacto-D-mannan. These were fractionated with Fehling solution, which precipitated the galactomannan as the copper complex. The soluble α-D-glucan (2% yield) had a specific rotation of +243°. Its ^{13}C-n.m.r. spec-

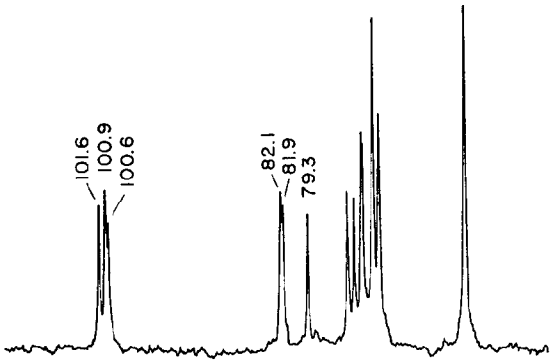


Fig. 3. ^{13}C-n.m.r. spectrum of isolichenan (from *C. islandica*) in deuterium oxide at 70°. Numerical values are δ in p.p.m.

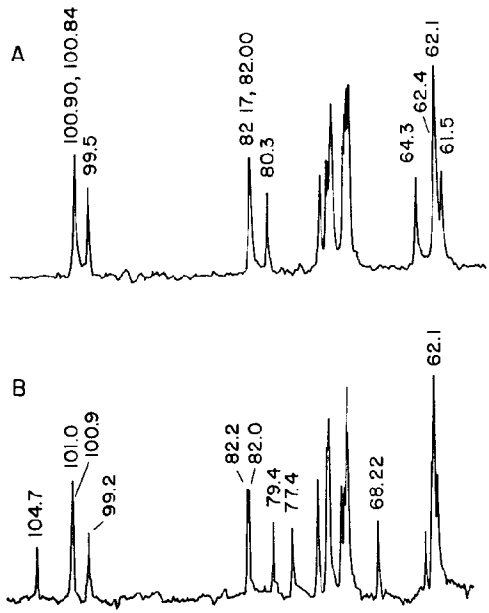


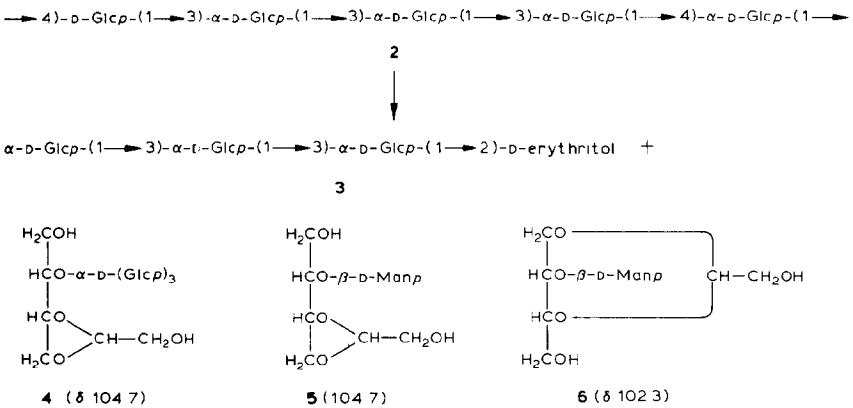
Fig. 4. ^{13}C-n.m.r. spectra, for solutions in deuterium oxide at 70°, of: (A) α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→2)-D-erythritol (**3**), and (B) *O*-hydroxyethylidene derivative **4**. Numerical values are δ in p.p.m.

trum (Fig. 2B) showed similarities to that of isolichenan* (Fig. 3), although a larger signal at δ 101.0 and a smaller one at δ 79.3 indicated some structural differences. Methylation analysis (Table I) indicated that (1→3) and (1→4) linkages were pre-

*The ^{13}C-n.m.r. spectrum of isolichenan (Fig. 3) is of interest since the ratio of the signals for O-3 and -4 substitutions at δ 82.1, 81.9, and 79.3, respectively, is 33:17, indicating a higher proportion of 3-*O*-substituted residues than the values of 60 (ref. 2) and 55% (ref. 5) that were previously recorded.

sent as 69% and 22%, respectively, of the total structures. A small proportion of branching was present with nonreducing end-groups (4%), and 3,4-(3%) and 2,3-di-*O*-substituted (2%) residues. The (1→3)- and (1→4) linkages of the D-glucan are distributed regularly along the linear chain in view of the Smith degradation data.

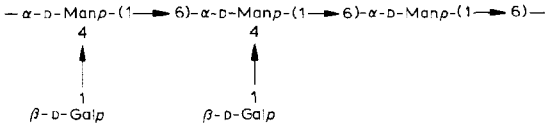
Following mild acid hydrolysis of the Smith-oxidized polyol, a product was obtained that gave two main spots on a paper chromatogram. The mixture was fractionated by cellulose column chromatography giving, in the order of emergence, three products in ratios of 17:30:4. The major component had a specific rotation of +177° and gave glucose and erythritol on hydrolysis; methylation analysis showed that glucopyranosyl units were present as nonreducing ends and 3-*O*-substituted units in a 1:2 ratio. These data are consistent with an *O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→2)-D-erythritol structure (**3**). Corresponding with the structure, the ^{13}C-n.m.r. spectrum (Fig. 4) showed three signals for C-1 at δ 100.9, 100.8, and 99.5; three signals corresponding to *O*-substituted carbon atoms at δ 82.2, 82.0, and 80.3; and five signals corresponding to carbon atoms linked to unsubstituted primary hydroxyl groups at δ 64.3, 62.4, 62.1 (two signals), and 61.5. The other main Smith-degradation product appeared to be an *O*-hydroxyethylidene derivative **4** of **3** (see Scheme 1), suggesting for the parent D-glucan the principal repeating structure **2**, which resembles the D-glucan previously isolated[18] from the lichen *Stereocaulon japonicum*. The ^{13}C-n.m.r. spectrum of the *O*-hydroxyethylidene derivative **4**, showed signals for C-1 and *O*-substituted carbon atoms of the triglucosyl residue, at δ 104.7 for C-1 of the hydroxyethylidene residue, and at δ 68.2 and 77.4 for *O*-substituted carbon atoms (Fig. 4B). The positions of substitution of the acetal group in the erythritol residue were determined by comparison of the chemical shift of the hydroxyethylidene C-1 (δ 104.7) with the shifts of known, structurally related derivatives[19] of 2-*O*-β-D-mannopyranosyl-D-erythritol being substituted by an hydroxyeth-



Scheme 1. Smith degradation of α-D-glucopyranan of *R. usnea* (**2**→**3**), and by-products, **4**, **5** and **6** from a β-D-mannopyranan containing (1→3) and (1→4) linkages.

ylidene group in the erythritol residue with 5- (**5**; δ 104.7) and 6-membered (**6**; δ 102.3) rings. Although a more substantial difference was expected between the resonances of the acetal carbon atoms of 5- and 6-membered rings (~10 p.p.m.)[20–24], the exact correspondence of the acetal resonance of **4** with that of a 5-membered ring is sufficient to assign the structure.

A *R. usnea* polysaccharide giving an insoluble copper complex was obtained in 2% yield; it contained galactose, mannose, and glucose in ratios of 43:53:3; and it had a specific rotation of +63°. The mannosyl and galactosyl units existed as the D enantiomers. Methylation analysis (Table I) showed the presence of nonreducing D-galactopyranosyl (40%), D-mannopyranosyl (2%), and D-galactofuranosyl (3%) end-groups, and 6-*O*- (19%) and 4,6-di-*O*-substituted (37%) D-mannopyranosyl residues. Partial hydrolysis of the polysaccharide provided a resistant core having a specific rotation of +61° and containing 17% of D-galactose and 83% of D-mannose. According to methylation studies, it consisted mainly of (1→6)-linked mannopyranosyl residues (66%) with nonreducing D-mannopyranosyl (11%) and D-galactopyranosyl (14%) end-groups, and 4,6- (7%) and 2,6-di-*O*-substituted (2%) D-mannopyranosyl residues. The ^{13}C-n.m.r. spectrum (Fig. 2C) of the D-galacto-D-mannan indicated the presence of D-galactopyranosyl groups in the β form (C-1, δ 104.8). According to the aforementioned data, these groups are linked (1→4) to



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the main chain; thus, a high proportion of structure **7** is present with substituents on approximately two of every three (1→6)-linked α-D-mannopyranosyl residues of the main-chain. Thus, the D-galacto-D-mannans from *C. islandica* and *R. usnea,* which both have fruticose-growth forms, have related but different chemical structures. Whether most lichens, which have ascomycetous mycosymbionts, contain such galactomannans is of significance. Also the sources of this and other polysaccharides are of interest, whether they arise from the fungal or algal component of each lichen.

EXPERIMENTAL

^{13}C-N.m.r. spectroscopy. — ^{13}C-N.m.r. spectra were recorded with a Varian XL-100-15 spectrometer in the Fourier-transform mode for solutions in deuterium oxide (0.85 mL) of the compound (10–100 mg) contained in a coaxial glass cylinder fitting snugly within a tube (0.12 diam. × 20.3 cm) maintained at 70°. The spectral width was 5000 Hz, the acquisition time 0.8 s, the pulse width 9.5 μs, and the number of transients 30 000 to 150 000 depending on the size of the sample

(100–10 mg). Chemical shifts are expressed in δ relative to the resonance of Me_4Si, obtained in a separate experiment.

Sugar composition of polysaccharides. — Polysaccharide was hydrolyzed with 0.5M sulfuric acid for 18 h at 100°, the solution made neutral ($BaCO_3$) and filtered, and the filtrate evaporated. The residue was converted into hexitol hexacetates by successive sodium borohydride reduction and acetylation with acetic anhydride–pyridine at 100°. These mixtures were analyzed[25] 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).

Methylation analysis of polysaccharides. — The samples (~10 mg) was methylated successively by the methods of Haworth[26] and Kuhn *et al.*[27]. The methylated polysaccharides were converted into partially methylated alditol acetates by successive treatments with 5% methanolic hydrogen chloride for 5 h at reflux and 10% aqueous sulfuric acid for 18 h at 100°, reduction of the product with sodium borohydride, and acetylation with acetic anhydride–pyridine. The acetates were examined by g.l c. in a conventional column (120 × 0.4 cm i.d.) of 3% (w/w) ECNSS-M on Chromosorb W (80–100 mesh), from 120 to 170° (4°/min, then hold). G.l.c.–m.s. was performed with a Model 4000 Finnigan unit, interfaced with an Incos 2300 Data System, and equipped with a capillary column (0.25 mm i.d. ×30 m) coated with 3:1 OV-225–OV-17. Electron-impact spectra were obtained repetitively every 2 s, scanning from 40 to 420. Injections were made in the splitless mode at 50° and a rapid program (40°/min) to 220° (hold) was carried out. The carrier gas was helium (linear velocity, 22 cm/s). Final identification of *O*-methylalditol acetates was obtained by co-injection with standards. Results are presented in Table I.

In the characterization by g.l.c.–m.s. of 3-*O*-methylmannitol pentaacetate from *C. islandica* galactomannan, the result was ambiguous, since this compound is identical with the 4-*O*-methyl isomer. To distinguish the two types of substitution, the free *O*-methylated hexose mixture was treated with 3% methanolic hydrogen chloride at reflux for 3 h, and the product acetylated with acetic anhydride–pyridine. By use of the ECNSS-M column, a peak was obtained corresponding to methyl 2,4,6-tri-*O*-acetyl-3-*O*-methylmannoside (retention time 10.2 min) rather than the 4-*O*-methyl isomer (retention time 11.6 min).

Preparation of D-*galacto*-D-*mannan-containing fraction from* C. islandica *(Fraction A).* — Iceland moss (200 g), obtained from S. S. Penick and Co. (New York), was treated with boiling water (3 L) for 1 h and, after cooling to 4°, the suspended mixture of lichen and lichenan was strained through cheese cloth. Insoluble material was suspended in cold water (2 L), and the suspension was again strained. This process was repeated and the combined supernatant solutions evaporated to give a solution (750 mL) which was cooled to 4° and recentrifuged to remove more lichenan. The supernatant solution was frozen and then slowly thawed at 4°. Following centrifugation of the precipitate, the solution was added to excess ethanol (3 L), the insoluble polysaccharide collected and dissolved in water (100 mL), Fehling solution (400 mL) added, and the precipitate filtered off and washed succes-

sively with aqueous 2% potassium hydroxide and methanol. The copper complex was decomposed by shaking in an aqueous suspension of Amberlite IR-120 (H^+), which was filtered off, and the filtrate concentrated to 10 mL. Excess ethanol was added to give a precipitate (Fraction A, 1.30 g), $[\alpha]_D^{25}$ +88° (*c* 0.5, water), that contained galactose, mannose, and glucose in the ratios of 40:43:17.

Cellulase treatment of Fraction A. — A solution of Fraction A (1.15 g) in water (50 mL) was treated for 18 h at 39° with cellulase Type 1 from *Aspergillus niger* (1.0 g, Sigma); having an activity of 1.3 μU/mg (liberating 1.0 μmol of D-glucose from cellulose at pH 5.0 in 1 h at 37°). After 18 h, the product was isolated by precipitation with excess ethanol, and the precipitation step with Fehling solution repeated. The product (Fraction B, 0.75 g) had $[\alpha]_D^{25}$ +91° (*c* 0.5, water) and contained galactose, mannose, and glucose in ratios of 45:49:6.

Partial hydrolysis of C. islandica D-*galacto*-D-*mannan B.* — Fraction B (0.11 g) was treated for 3 h with 0.16M sulfuric acid (11 mL) at 100°. The hydrolyzate was made neutral ($BaCO_3$), filtered, and concentrated to 0.5 mL. Polysaccharide (41 mg) obtained by precipitation with excess ethanol had $[\alpha]_D^{25}$ +81° (*c* 0.8, water), and contained galactose, mannose, and glucose in ratios of 30:66:4. The supernatant solution contained the liberated hexoses in ratios of 81:12:7.

Most of the galactosyl groups could be removed by partial hydrolysis over 19 h to give Fraction C (yield 16 mg); $[\alpha]_D^{25}$ +51° (*c* 0.5, water); ratios of galactose, mannose, and glucose 21:74:5; methylation data of the acid-degraded polysaccharide are presented in Table I; ^{13}C-n.m.r. (D_2O, 70°): δ 101.0, 72.5 (2 signals), 71.6, 68.5, and 67.5 corresponding to (1→6)-linked α-D-mannopyranan[11], and 62.8 (C-6 of nonreducing galactopyranosyl and mannopyranosyl end-groups).

Partial acetolysis of Fraction A. — The polysaccharide (0.70 g) was partially acetolyzed according to the method of Lee and Ballou[28]. The product (reducing sugars) was chromatographed on a column of cellulose in 7:1 (v/v) acetone–water to yield 2-*O*-α-D-galactopyranosyl-D-mannose (44 mg), $[\alpha]_D^{25}$ +70° (*c* 0.4, water); lit.[29] $[\alpha]_D$ +76°; ^{1}H-n.m.r. (D_2O): δ 5.32 (*J* 0.3 Hz, H-1′), 5.03 (*J* 1.0 Hz, H-1, α anomer); ^{13}C-n.m.r. (D_2O, 70°): δ 102.9 (C-1′), 94.4 (C-1), and 81.9 (C-2); both spectra identical with those of known materials[29]. Hydrolysis gave galactose and mannose. On hydrolysis of borohydride-reduced material only galactose was obtained. The disaccharide was resistant to oxidation with lead tetraacetate in acetic acid indicating a (1→2) linkage[30]. Methylation analysis gave the acetates of 2,3,4,6-tetra-*O*-methylgalactitol and 3,4,6-tri-*O*-methylmannitol.

Smith degradation of C. islandica D-*galacto*-D-*mannan Fraction B.* — Fraction B (160 mg) was oxidized in a solution of sodium metaperiodate (500 mg) in water (20 mL) for 3 days. The solution was then de-ionized and reduced with sodium borohydride. The product was partially hydrolyzed in water (10 mL) adjusted to pH 2 with aqueous sulfuric acid for 30 min at 100°. Following de-ionization of the solution, it was chromatographed on paper in 40:11:19 (v/v) butanol–ethanol–water (spray: ammoniacal silver nitrate), showing the presence of glycerol, erythritol (traces), and two spots having R_{Man} 0.85 and 0.5. Cellulose-col-

umn chromatography of the mixture with 10:1 acetone–water gave glycerol (45 mg), with 7:1 acetone–water erythritol (2 mg) and the material with R_{Man} 0.85 (32 mg), and with 4:1 (all v/v) acetone–water the material with R_{Man} 0.5 (4 mg). The compound of R_{Man} 0.85 corresponds to 1-*O*-α-D-mannopyranosyl-L-glycerol, $[\alpha]_D^{25}$ +53° (*c* 0.4, water), and gave mannose and glycerol on hydrolysis; ^{13}C-n.m.r. (D_2O, 70°): δ 101.5 (C-1), 74.5, 72.3, 71.9, 71.7, 70.0, 68.6, 64.3 (C-3), and 62.7 (C-6′).

Ethanol extraction of R. usnea. — *R. usnea* (90 g) was collected from orchard trees growing near Quatro Barros, Parana, Brazil, near the foot of the Serra do Mar, on the land side, and was extracted with 2:1 (v/v) benzene–ethanol (2 L) for 2 h at reflux. A suspension of the remaining insoluble material (88 g) in water (1 L) was boiled and the extract decanted. This process was repeated two more times, and the combined extracts were concentrated to 200 mL. The solution was frozen, and thawed slowly at 4°, and the insoluble material (Fraction D, 0.40 g) collected. The mother liquor was concentrated to 50 mL and the polysaccharide (Fraction E, 6.50 g) precipitated by addition of ethanol (5 vol.). The mother liquor contained polyols (Fraction F, 1.32 g).

Fractionation of ethanol-soluble materials R. usnea, *Fraction F.* — Fraction F was acetylated and examined by g.l.c.–m.s. in a capillary column of 3:1 OV-225–OV-17. The peaks obtained corresponded to glycerol (6%), erythritol (1%), unknowns (12%), arabinitol (74%), and xylitol (8%). A portion (0.75 g) of Fraction F was chromatographed on a column of cellulose in 7:1 (v/v) acetone–water. A pentitol fraction (0.39 g) was obtained, and crystallization from ethanol gave D-arabinitol, m.p. 101–103°, $[\alpha]_D^{25}$ +10° (*c* 0.5, satd. disodium tetraborate in water). Elution with 4:1 (v/v) acetone–water gave material (65 mg) having, on paper in 2:1:1 (v/v) 1-butanol–ethanol–water, $R_{lactose}$ 0.8.

Fractionation of α-D-*glucan and* D-*galacto*-D-*mannan of* R. usnea. — A portion of water-soluble polysaccharides (Fraction E, 1.2 g) from *R. usnea* was treated with Fehling solution as outlined earlier. The galactomannan **7** (Fraction G, 0.35 g), regenerated from the insoluble copper complex, had $[\alpha]_D^{25}$ +63° (*c* 0.3, water), and contained galactose, mannose, and glucose in ratios of 43:53:3. Methylation data are presented in Table I.

The glucan (**2**) was present in the supernatant solution and, following neutralization with acetic acid, the solution was dialyzed and then de-ionized to remove last traces of salt (yield 0.27 g), $[\alpha]_D^{25}$ +243° (*c* 0.4, water). It was analyzed by the methylation technique (see Table I).

Partial hydrolysis of R. usnea D-*galacto*-D-*mannan.* — By use of the same partial-hydrolysis time (19 h) and conditions outlined for the degradation of Fraction A, a product (11% yield) was obtained, $[\alpha]_D^{25}$ +61° (*c* 0.2, water), having a galactose-to-mannose ratio of 17:83.

Smith degradation of R. usnea α-D-*glucan.* — α-D-Glucan (100 mg) was oxidized for 3 days in excess aqueous sodium periodate, the product reduced with sodium borohydride, and the polyol partially hydrolyzed at pH 2 and 100°, for 30

min, as outlined above. On a paper chromatogram in 2:1:1 (v/v) butanol–ethanol–water (spray: ammoniacal silver nitrate), three spots were obtained with mobilities, relative to that of 2-*O*-α-D-glucopyranosyl-D-erythritol (R_{GE}), of 0.5, 0.3, and 0.2. Cellulose-column chromatography in 4:1 (v/v) acetone–water gave a material with R_{GE} 0.5 (17 mg), and a 3:1 solvent ratio compounds with R_{GE} 0.3 (30 mg) and 0.2 (4 mg).

On hydrolysis, the compound having R_{GE} 0.3, $[\alpha]_D^{25}$ +177° (*c* 0.5, water), gave glucose and erythritol, and, on methylation analysis, 2,3,4,6-tetra- and 2,4,6-tri-*O*-methylglucitol acetates in a 1:2 ratio; the ^{13}C-n.m.r. spectrum (Fig. 4A) corresponds to structure **3**.

The material having R_{GE} 0.5 (**4**), $[\alpha]_D^{25}$ +155° (*c* 0.3, water), showed ^{13}C-n.m.r. spectrum (Fig. 4B), resembling that of structure **3**, but with additional signals at δ 104.7, 68.2 and 77.4. The ^{13}C-n.m.r. spectrum of the *O*-hydroxyethylidene derivative **5** showed signals at δ 102.3 (acetal C) and 101.6 (C-1′), and that of *O*-hydroxyethylidene derivative **6** related signals at δ 104.6 (acetal C) and 101.8 (C-1′).

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