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Polysaccharides of lichenized fungi of three *Cladina* spp.: significance as chemotypes

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Abstract

The chemical structures of nigerans, an α-glucan, laminarans, a β-glucan, galactoglucomannans and galactomannoglucans of three species of *Cladina*, namely *C. arbuscula*, *C. confusa* and *C. substenius*, were determined and compared. According to chemical and spectroscopic analyses, the glucans investigated to date, which have been isolated in 15 *Cladonia* species, as well as the galactomannoglucans and galactoglucomannans, have similar principal structures. This suggests that, in terms of polysaccharide structure, *Cladonia* and *Cladina* genera do not show differences. DNA studies support this idea and it is proposed that *Cladina* be reduced to a synonym under *Cladonia*.

Keywords: Lichenized fungi; *Cladina* spp.; Chemotaxonomy; Polysaccharides; Structure

1. Introduction

Of the 13,500 lichenized fungi species growing worldwide, no more than 100 species have been investigated for their carbohydrate compositions. The best known polymers of lichens are lichenan, isolichenan, galactomannan, and the recently described galactomannoglucans (Woranovicz-Barreira et al., 1999b; Carbonero et al., 2001), which can be useful in chemotaxonomic studies (Gorin et al., 1993; Teixeira et al., 1995; Woranovicz-Barreira et al., 1999b). In lichenized fungi, large genera are usually divided into subgenera and then again into sections, which include very closely related species. According to classic taxonomy, *Cladina* was a subgenus of the *Cladonia* genus and thereafter lichenologists decided to consider it as a distinct genus. On the basis of molecular phylogenetic results, Ahti and Depriest (2001) proposed that lichens currently placed in the segregated genus *Cladina* be transferred to the genus *Cladonia*, and that *Cladina* becomes a synonym of *Cladonia*. Studies on polysaccharide components showed that nigeran occurred in several *Cladonia* spp. (Nishikawa et al., 1974; Woranovicz-Barreira et al., 1999a), and also in four *Cladina* spp. (Nishikawa et al., 1974; Iacomini et al., 1985). Related galactoglucomannans were also found in these separate genera. In this study, β-D-Glucans and galactomannoglucans, which were suggested as markers for lichen identification in the *Cladonia* genus (Woranovicz-Barreira, 1999a; Carbonero et al., 2001), were found in the three *Cladina* spp. examined. These polysaccharides are thus useful chemical markers in lichen identification and for confirmation of DNA studies.

2. Results and discussion

The extracts of each lichen species, obtained by treatment with organic solvents and 10% aq. KOH at 100 °C, were neutralized, treated with excess ethanol and the precipitate obtained was resuspended in water, frozen, and then thawed, resulting in the formation of a precipitate (FT1) and soluble material (FT2) (Scheme 1). FT1 was resuspended with 0.5% aq. KOH at 50 °C,

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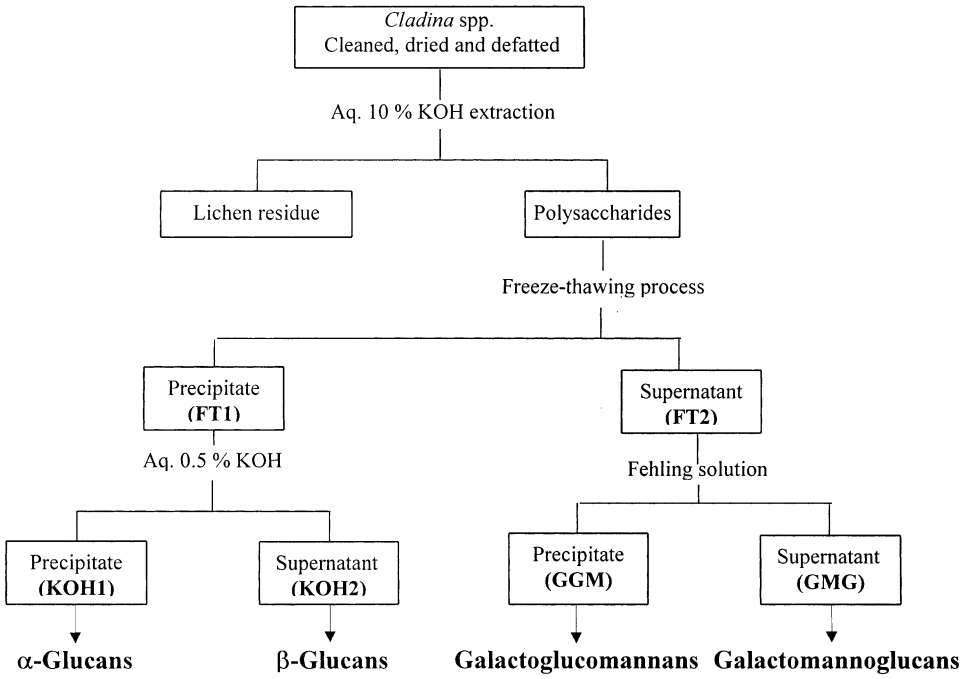

Scheme 1. Extraction and purification of polysaccharides of *Cladina* spp.

which also gave two fractions, one insoluble (KOH1) and the other soluble (KOH2) under these conditions.

2.1. (1→3),(1→4)-Linked α-glucans (KOH1)

Products with a high content of glucose were obtained (FT1, Table 1). After purification, each KOH1 fraction from the three species (yields ~2%) gave rise to ^{13}C NMR spectra with 12 signals (Fig. 1; Table 2), arising from an α-D-glucan with alternating (1→3)- and (14)-linkages in a 1:1 ratio, previously found in various *Cladonia* spp. (Woranovicz-Barreira et al., 1999a; Carbonero et al., 2001), and also in four species of the *Cladina* genus (Iacomini et al., 1985; Nishikawa et al., 1974). Many other α-glucans, isolated using the freeze-thawing process, have been studied. Except for that of *Flavoparmelia caperata*, all displayed different (1→3)- and (1→4)-linkage ratios, varying between 2:1 and 3:1 (Gorin et al., 1988). We thus suggest that the 1:1 ratio and other parameters could be used as a marker to aid lichen taxonomy. To check whether this polysaccharide is a "fingerprint" in *Flavoparmelia* spp., other species should be investigated, considering that only one has been studied so far.

2.2. (1→3)-Linked β-glucans (KOH2)

β-D-Glucans of the laminaran type are polysaccharides insoluble in cold water and are formed as a precipitate in the freeze-thawing process, arising from 15 *Cladonia* spp. (Carbonero et al., 2001). This indicates that they should be present in all lichens of this genus. Indeed, the freeze-thawing process carried out on extracts of three *Cladina* spp. gave rise to identical glucans in the KOH2 fraction of the aq. 0.5% KOH treatment (yield ~0.4%). Their ^{13}C NMR spectra (Fig. 2;

Table 1
Monosaccharide composition and yields of polysaccharides obtained from *Cladina* spp.

Lichen	Freeze-thawing treatment				Fehling treatment			
	Precipitate (FT1)		Supernatant (FT2)		Precipitate (GGM)		Supernatant (GMG)	
	(%)[b] Man:Gal:Glc	Yield (%)[b]	(%)[a] Man:Gal:Glc	Yield (%)[b]	(%)[a] Man:Gal:Glc	Yield (%)[b]	(%)[a] Man:Gal:Glc	Yield (%)[b]
C. arbuscula	2:0:98	2.5	63:32:5	8.5	57:40:3	3.2	41:47:12	1.5
C. confusa	1:1:98	3.1	60:32:8	15.3	63:32:5	11.0	40:49:11	3.4
C. substenius	0:1:99	1.6	58:36:6	10.9	55:41:4	3.2	47:40:13	3.4

[a] Derived alditol acetates analyzed by GC–MS.
[b] Yields based on dry lichen.

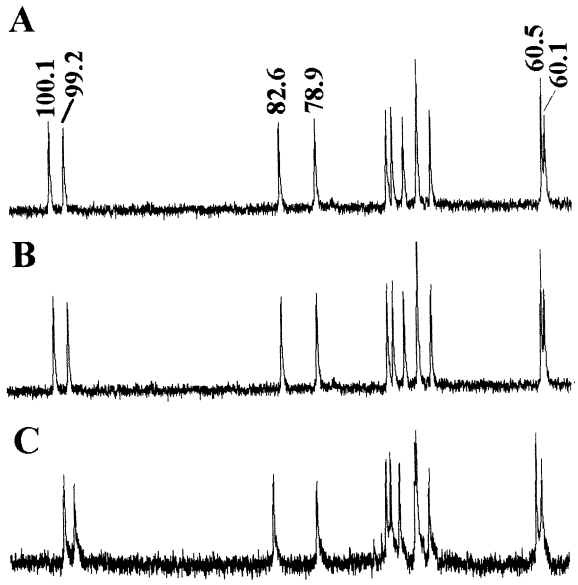

Fig. 1. ^{13}C NMR spectra of nigerans (KOH1) in Me_2SO-d_6 at 70 °C (chemical shifts are expressed as δ ppm) obtained from *Cladina arbuscula* (A), *C. confusa* (B), and *C. subtenius* (C).

Table 2
Proton and ^{13}C chemical shifts (δ) of nigerans (KOH1) and laminarans (KOH2), obtained from *Cladina* spp.

$^1H/^{13}C$ nuclei	KOH1				KOH2	
	(1→4)-α-Glc		(13)-α-Glc		(1→3)-β-Glc	
	^1H (δ)	^{13}C (δ)	^1H (δ)	^{13}C (δ)	^1H (δ)	^{13}C (δ)
H-1/C-1	5.23	100.1	5.14	99.2	4.55	102.9
H-2/C-2	3.55	70.6	3.48	71.9	3.31	72.9
H-3/C-3	3.73	72.6	3.70	82.6	3.49	86.2
H-4/C-4	3.57	78.9	3.52	69.5	3.27	68.4
H-5/C-5	3.88	73.1	4.03	70.5	3.27	76.3
H-6/C-6	3.63	60.5	3.76	60.1	3.48/3.72	60.8

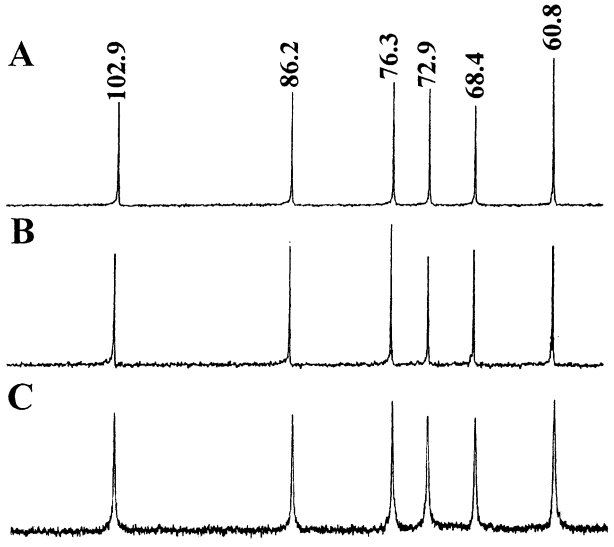

Fig. 2. ^{13}C NMR spectra of laminaran (KOH2) in Me_2SO-d_6 at 70 °C (chemical shifts are expressed as δ ppm) obtained from *Cladina arbuscula* (A), *C. confusa* (B), and *C. subtenius* (C).

Table 2) containing six signals of equal magnitude, are identical to that of an authentic (1→3)-linked β-glucan. These were also obtained in low yields from *Stereocaulon ramulosum* (Baron et al., 1988) and *Ramalina celastri* (Stuelp et al., 1999). One common factor in the analyzed lichens is that they all contain *Trebouxia* sp. (family Chlorophyceae) as a photobiont, suggesting that it might be the source of this β-glucan and can be used as a marker to aid the classic taxonomy of lichenized fungi. This polysaccharide has also been proposed as a morphological characteristic of cladoniform lichens (Gorin et al., 1988; Stenroos and Depriest, 1998).

2.3. Galactoglucomannans (GGM)

The GGM fraction was obtained via Fehling precipitation from FT2. Heteropolysaccharides from *C. arbuscula* (yield 3.2%), *C. confusa* (yield 11.0%) and *Cladina substenius* (yield 3.2%) each having M_r 1.9×10^6, and mannose, galactose and glucose in ratios of 57:40:3, 63:32:5, and 55:41:4 respectively, were superficially similar. Their ^{13}C NMR spectra (Fig. 3) showed major signals in common, but had minor differences typical of the species. In general, we have found that such ^{13}C NMR spectra correspond to the lichen species (Woranovicz et al., 1997, 1999), to the extent that they were used for classification and identification (Teixeira et al., 1995).

Methylation analysis showed complex structures with formation of at least 10 significant, partially *O*-methylated alditol acetates (Table 3), which indicates structures with similarities to those of heteropolysaccharides of the *Cladonia* and *Cladina* spp. (Iacomini et al., 1985; Woranovicz et al., 1997, 1999) [this complexity was reflected in the ^{13}C NMR spectra, which contain many signals in the C-1 region (Fig. 3)]. These signals corresponded, according to the methylation data (Table 3), to nonreducing end-groups of Gal*p* and Man*p* (*C. confusa* showed the highest percentage of Man*p*, 20%, among in the three species), and Man*p* units substituted at O-2, O-6, O-4,6 (in this case, the *C. confusa* polymer had the lowest content), O-2,6 and O-2,4,6. Moreover, the *C. confusa* polysaccharide presented Gal*p* units substituted at O-3, differ from *C. arbuscula* and *C. substenius*, which had them substituted at O-4.

The ^{13}C NMR spectra of the three GGM (Fig. 3), contained C-1 signals that indicated predominant branched structures with nonreducing end-units of β-D-Gal*p*-(1→4)-α-D-Man*p* (δ 104.3), α-D-Gal*p*-(1→2)-α-D-Man*p* (δ 102.8), and also α-D-Man*p* (δ 103.3) linked (1→2) to α-D-Man*p* units, along with 2,4-di-*O*-substituted (δ 101.7) (Gorin and Iacomini, 1984, 1985), 6-*O*- (δ 100.5) and 2,6-di-*O*-substituted (δ 99.3) units of α-D-Man*p* from the polysaccharide core (Gorin, 1973).

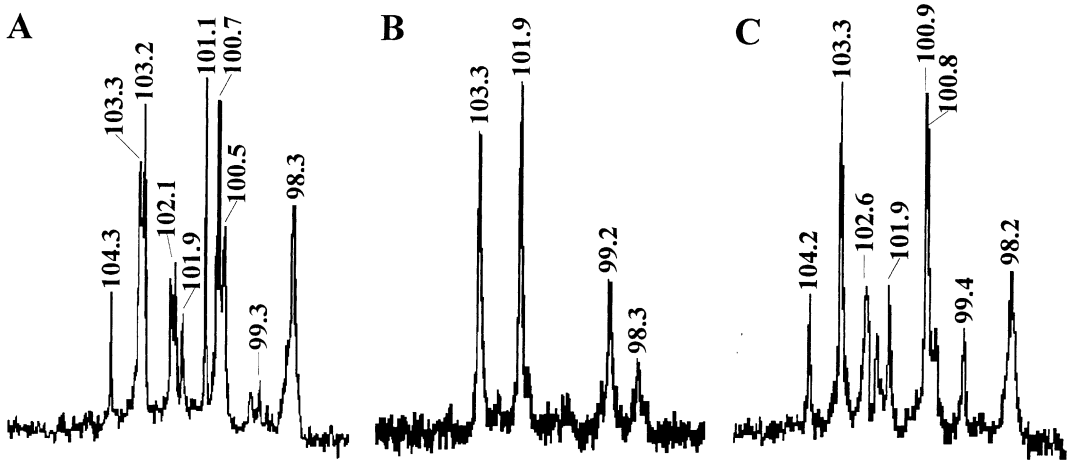

Fig. 3. ^{13}C NMR spectra of the galactoglucomannans (GGM) in D_2O at 30 °C (chemical shifts are expressed as δ ppm), obtained from *Cladina arbuscula* (A), *C. confusa* (B), and *C. subtenius* (C).

Table 3
Linkage types based on analysis of partially *O*-methyl alditol acetates obtained from methylated galactoglucomannans (GGM) isolated from *Cladina* spp. [a]

Linkage type[b]	GGM		
	C. arbuscula	*C. confusa*	*C. substenius*
Man*p*-(1→	8	20	11
Gal*p*-(1→	29	24	27
→2)-Man*p*-(1→	9	4	7
→3)-Glc*p*-(1→	–	1	–
→4)-Man*p*-(1→	3	2	1
→3)-Gal*p*-(1→	–	6	–
→6)-Man*p*-(1→	5	5	7
→4)-Gal*p*-(1→	11	2	14
→4)-Glc*p*-(1→	3	4	4
→4,6)-Man*p*-(1→	12	4	11
→2,6)-Man*p*-(1→	9	12	7
→2,4,6)-Man*p*-(1→	11	16	11

[a] % Of peak area of *O*-methylalditol acetate relative to total area (GC–MS).
[b] Based on derived *O*-methylalditol acetates.

2.4. Galactomannoglucans (GMG)

The mother liquors, obtained after Fehling precipitation, gave similar homogeneous polysaccharides (yields: 1.5% in *C. arbuscula* and 3.4% for the two other species, M_r 1.75×10^6). They have similarities in their ^{13}C NMR spectra (Fig. 4) and Man, Gal, Glc ratios of 41:47:12 (*C. arbuscula*), 40:49:11 (*C. confusa*), and 47:40:13 (*C. substenius*), respectively.

The ^{13}C NMR spectra of each GMG showed a complex side-chain structure with C-1 signals of β-Gal*f* at δ 106.0–108.3 (Gorin et al., 1981). This agreed with the methylation data, which showed 16–26% of Gal*f* non-reducing end units (Table 4). This analysis also showed that the side-chains were branched (with 2-*O*-, 4-*O*-, 6-*O*-, 2,3-di-*O*-substituted Man*p*; 6-*O*-, 3,6-di-*O*-substituted Gal*p* units; and 2,3,6-tri-*O*-substituted Glc*p* units) being present in all investigated *Cladina* spp. These results coincide with those obtained for several species of the genus *Cladonia* (Woranovicz-Barreira et al., 1999b), which gave rise to virtually identical ^{13}C NMR spectra and methylation data, i.e. galactomannoglucans with (1→3)-linked main chains of β-D-Glc*p*, substituted at O-2,6 principally by side chains of Gal*f* and Man*p* units.

In contrast, the only galactan isolated from this fraction was obtained from a *Cladina stellaris* fraction (Iacomini et al., 1985). Perhaps this species should be further studied, as *Cladina confusa* also contained a galactan, but no galactomannoglucan. *Cladia aggregata*, which belongs to the same family, did not have this heteropolysaccharide (data not shown). It is known that it is not related to the genus *Cladonia* as is *Cladina*. The present data suggest that the *Cladonia* and *Cladina* genera have the same heteropolymer contained in the Fehling supernatant.

3. Conclusion

These results, along with DNA studies (Ahti and Depriest, 2001), now suggest that *Cladina* must be classified within the genus *Cladonia*.

4. Experimental

4.1. Lichenized fungi

Cladina arbuscula (Wallr.) Hale & W. L. Culb. was collected in Finland, Province of Uusimaa. *Cladina confusa* (Sant.) Follm. & Ahti, from the Ilha do Mel, State of Paraná, Brazil, and *Cladina substenius* (Abbayes) Hale & W. L. Culb. in Massachusetts, USA. The three samples were collected in 1998, and have their vouchers deposited in the UPCB (Herbarium of the

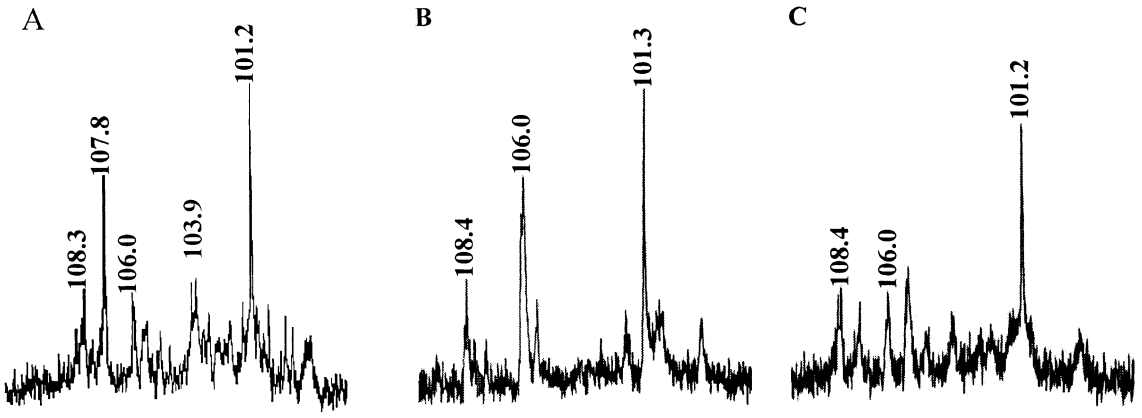

Fig. 4. ^{13}C NMR spectra of the galactomannoglucans (GMG) in D_2O at 30 °C (chemical shifts are expressed as δ ppm), obtained from *Cladina arbuscula* (A), *C. confusa* (B), and *C. subtenius* (C).

Table 4
Linkage types based on analysis of partially *O*-methyl alditol acetates obtained from methylated galactomannoglucans (GMG) isolated from *Cladina* spp.[a]

Linkage type[b]	GMG		
	C. arbuscula	*C. confusa*	*C. substenius*
Man*p*-(1→	5	3	4
Galf-(1→	22	26	16
Gal*p*-(1→	10	9	12
→2)-Man*p*-(1→	16	9	19
→2)-Glc*p*-(1→	3	2	4
→3)-Man*p*-(1→	–	3	–
→4)-Man*p*-(1→	7	6	11
→6)-Man*p*-(1→	2	5	6
→6)-Galf-(1→	7	6	6
→2,3)-Man*p*-(1→	7	8	5
→3,4)-Gal*p*-(1→	3	–	–
→4,6)-Man*p*-(1→	4	2	1
→2,6)-Man*p*-(1→	–	2	2
→4,6)-Gal*p*-(1→	–	3	2
→3,6)-Gal*p*-(1→	5	5	4
→2,3,6)-Glc*p*-1→	10	9	9

[a] % Of peak area of *O*-methylalditol acetate relative to total area (GC–MS).
[b] Based on derived *O*-methylalditol acetates.

Federal Universsity of Paraná), registration numbers 45901, 35700, 45900, respectively.

4.2. General analytical methods

Evaporations were carried out under reduced pressure at 30 °C. Gas liquid chromatography–mass spectrometry (GC–MS) was performed using a Varian model 3300 gas chromatograph linked to a Finnigan Ion-Trap, model 810 R-12 mass spectrometer, with a DB-225 capillary column (30×0.25 mm i.d.) held at 50 °C for injection, and then programmed at 40 °C/min to 220 °C (constant temp), with He as carrier gas. Paper chromatography was carried out using Whatman No. 1 filter paper (solvent: *n*-BuOH–pyridine–H_2O, 5:3:3, v/v), with glucose as standard; sugars being detected by the acetone–$AgNO_3$ dip method (Trevelyan et al., 1950). The phenol–H_2SO_4 method (Dubois et al., 1956) was used for quantitative sugar determinations.

4.3. Isolation and purification of polysaccharides

Lichen samples (*C. arbuscula*, 36 g; *C. confusa*, 37 g; *C. substenius,* 80 g) were refluxed (2 h) successively in $CHCl_3$–MeOH (2:1 v/v) and 80% aq. MeOH, in order to extract low molecular components. The residual material (24.0 g, 26.5 g and 55.0 g for *C. arbuscula*, *C. confusa* and *C. substenius*, respectively) was then extracted three times with 10% aq. 10% aq. KOH (300 ml) containing a trace of $NaBH_4$ at 100 °C for 3 h. The combined extracts were neutralized with HOAc, dialyzed against tap water for 72 h, and the retained solution was frozen and thawed to give a precipitate (FT1) and supernatant (FT2). The FT1 fraction (*C. arbuscula*, 0.9 g; *C. confusa*, 1.1 g; *C. substenius* 1.3 g) consisted of a mixture of (1→3), (1→4)-linked α-glucan and (1→3)-linked β-glucan, which was isolated and suspended with stirring in 0.5% aq. KOH at 50 °C, which dissolved the β- (KOH2), but not the α-glucan (KOH1). The FT2 fraction (*C. arbuscula*, 3.1 g; *C. confusa*, 5.7 g; *C. substenius* 8.7 g) was then treated with Fehling solution (Jones and Stoodley, 1965), and resulting precipitates of Cu complexes removed. Each fraction was neutralized with HOAc, dialyzed against tap water and deionized with mixed ion exchange resins, giving rise to galactoglucomannans (Cu^{++}-Ppt; GGM) and galactomannoglucan (Cu^{++}-Sup; GMG).

4.4. Monosaccharide composition

The polysaccharides were hydrolyzed with 1 M TFA at 100 °C for 8 h, followed by evaporation to dryness and successive reduction with $NaBH_4$ and acetylation with Ac_2O–NaOAc at 120 °C for 1 h. The Ac_2O was destroyed with ice-water, and the resulting alditol acetates extracted with $CHCl_3$ (Whiton et al., 1985) and analyzed by GC–MS, as described above.

4.5. Methylation analysis

Ten milligrams of each purified fraction, were per-*O*-methylated using powdered NaOH in Me_2SO–MeI (Ciucanu and Kerek, 1984). The per-*O*-methylated derivatives were treated with refluxing 3% HCl–MeOH for 2 h at 80 °C, then 0.5 M H_2SO_4 at 100 °C for 14 h, and the resulting mixtures of *O*-methyl aldoses, as described above, were then reduced with $NaBH_4$ and acetylated to give partially *O*-methylated alditol acetates, which were analyzed by GC–MS.

4.6. HPSEC analysis

The elution profiles of water-soluble fractions were determined by high performance size-exclusion chromatography (HPSEC), using a WATERS 510 HPLC pump at 0.6 ml/min with four gel permeation columns in series with exclusion sizes of 1×10^6, 4×10^5, 8×10^4, and 5×10^3 Da, using a refraction index (RI) detector. The eluent was 0.1 M/l aq. $NaNO_3$ containing 200 ppm aq. NaN_3. Samples, previously filtered through a membrane (0.22 μm; Millipore), were injected (250 μl loop) at 1 mg/ml concentration.

4.7. ^1H and ^{13}C nuclear magnetic resonance spectroscopy

1D ^1H, ^{13}C NMR spectroscopic experiments were obtained using a 400 MHz Brüker model DRX Avance spectrometer incorporating Fourier transform. ^{13}C NMR (100.6 MHz) and ^1H NMR (400.13 MHz) analyses were performed at 70 or 30 °C, with the samples being dissolved in D_2O or in Me_2SO-d_6 depending on their solubility. The OH groups were exchanged with D_2O followed by freeze drying. Chemical shifts of water-soluble samples are expressed in δ ppm relative to acetone at δ 30.20 and 2.22 for ^{13}C and ^1H signals, respectively and at δ 39.70 (^{13}C) and 2.40 (^1H) for those soluble in Me_2SO-d_6.

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References

Ahti, T., Depriest, P.T., 2001. New combination of *Cladina* epithets in *Cladonia* (Ascomycotina, Cladoniaceae). Mycotaxon. 78, 499–502.

Baron, M., Gorin, P.A.J., Iacomini, M., 1988. Isolation and identification of a linear (1→3)-linked β-D-glucan and other carbohydrate components of the lichen *Stereocaulon ramulosum* (Sw.). Räusch. Carbohydr. Res. 177, 235–239.

Carbonero, E.R., Sassaki, G.L., Stuelp, P., Gorin, P.A.J., Woranovicz-Barreira, S.M., Iacomini, M., 2001. Comparative studies of the polysaccharides isolated from lichenized fungi of the genus *Cladonia*: significance as chemotypes. FEMS Microbiol. Lett. 194, 65–69.

Ciucanu, I., Kerek, F., 1984. Simple and rapid method for the permethylation of carbohydrates. Carbohydr. Res. 131, 209–217.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Gorin, P.A.J., 1973. Rationalization of carbon-13 magnetic resonance spectra of yeast mannans and structurally related oligosaccharides. Can. J. Chem. 51, 2375–2383.

Gorin, P.A.J., Iacomini, M., 1984. Polysaccharides of the lichens *Cetraria islandica* and *Ramalina usnea*. Carbohydr. Res. 128, 119–132.

Gorin, P.A.J., Iacomini, M., 1985. Structural diversity of D-galacto-D-mannan components isolated from lichens having ascomycetous mycosymbionts. Carbohydr. Res. 142, 253–267.

Gorin, P.A.J., Baron, M., Iacomini, M., 1988. Storage products of lichens. In: Galun, M. (Ed.), CRC Handbook of Lichenology. CRC Press, Florida, pp. 9–23.

Gorin, P.A.J., Baron, M., da Silva, M.L.C., Teixeira, A.Z.A., Iacomini, M., 1993. Lichen carbohydrates. Ciência e Cultura (Brazil) 45, 27–36.

Gorin, P.A.J., Barreto-Bergter, E.M., da Cruz, F.S., 1981. Chemical structure of de D-galacto D-mannan component of *Trypanossoma cruzi*: C-13 NMR shift dependence on structure of D-mannose to D-galactose linkage. Carbohydr. Res. 88, 177–188.

Iacomini, M, Schneider, C.L., Gorin, P.A.J., 1985. Comparative studies on the polysaccharides of *Cladonia alpestris* (Reindeer moss), *Cladonia confusa*, and *Cladonia amaurocraea*. Carbohydr. Res. 142, 237–251.

Jones, J.K.N., Stoodley, R.J., 1965. Fractionation using copper complexes. Methods Carbohydr. Chem. 5, 36–38.

Nishikawa, Y., Ohki, K., Takahashi, K., Kurano, G., Fukuoka, F., Emori, M., 1974. Studies on the water soluble constituents of lichens. II. Antitumor polysaccharides of *Lasallia*, *Usnea*, and *Cladonia* species. Chem. Pharm. Bull. 22, 2692–2702.

Stenroos, S.K., Depriest, P.T., 1998. T.SSU rDNA phylogeny of Cladoniform lichens. Am. J. Bot. 85, 1548–1559.

Stuelp, P.M., Carneiro Leão, A.M.C., Gorin, P.A.J., Iacomini, M., 1999. The glucans of *Ramalina celastri*: relation with chemotypes of other lichens. Carbohydr. Polym. 40, 101–106.

Teixeira, A.Z.A., Iacomini, M., Gorin, P.A.J., 1995. Chemotypes of mannose-containing polysaccharides of lichen mycobionts: a possible aid in classification and identification. Carbohydr. Res. 266, 309–314.

Trevelyan, W.E., Procter, D.P., Harrison, J.S., 1950. Detection of sugars on paper chromatograms. Nature 166, 444–445.

Whiton, R.S., Lau, P., Morgan, S.L., Gilbart, J., Fox, A., 1985. Modifications in the alditol acetate method for analysis of muramic acid and other neutral and amino sugars by capillary gas chromatography-mass spectrometry with selected ion monitoring. J. Chromatogr. 347, 109–120.

Woranovicz, S.M., Gorin, P.A.J., Marcelli, M., Torri, G., Iacomini, M., 1997. Structural studies on the galactomannans of lichens of the genus *Cladonia*. The Lichenologist 29, 471–481.

Woranovicz, S.M., Pinto, B.M., Gorin, P.A.J., Iacomini, M., 1999. Novel structures in galactoglucomannans of the lichens *Cladonia substellata* and *Cladonia ibitipocae*: significance as chemotypes. Phytochemistry 51, 395–402.

Woranovicz-Barreira, S.M., Gorin, P.A.J., Sassaki, G.L., Tischer, C.A., Ahti, T., Iacomini, M., 1999a. Chemotyping studies on the glucans of lichens of the genus *Cladonia*. Phytochemistry 52, 1069–1074.

Woranovicz-Barreira, S.M., Gorin, P.A.J., Sassaki, P.L., Marcelli, M.P., Iacomini, M., 1999b. Galactomannoglucans of lichenized fungi of *Cladonia* spp.: significance as chemotypes. FEMS Microbiol. Lett. 181, 313–317.