ELSEVIER

Carbohydrate Research 266 (1995) 309–314

CARBOHYDRATE RESEARCH

Note

Chemotypes of mannose-containing polysaccharides of lichen mycobionts: a possible aid in classification and identification

Antonio Z.A. Teixeira, Marcello Iacomini, Philip A.J. Gorin *

Departamento de Bioquímica, Universidade Federal do Paraná, CP 19046, 81.531-990, Curitiba-PR., Brazil

Received 2 December 1993; accepted in revised form 30 June, 1994

Lichens consist of a predominant mycobiont(s), which is usually an ascomycete and less commonly a phyco-, basidio-, or deuteromycete [1], in association with a phycobiont, which can be a green alga, cyanobacterium, or both. The existence of 13250 species of ascomycetes capable of forming lichens has been claimed and these comprise 46% of all ascomycetes. Ascomycotina can be divided into ca. 2720 genera, much greater than the 37 reported for phycobionts of lichens [2]. This classification suggested the possible existence of a large number of structurally different mannose-containing polysaccharides of the mycobionts and their use as an aid in their classification and identification, by analogy with those of yeasts [3]. The ^{13}C NMR spectra of glucans of *Stereocaulon* spp. and other lichens have been suggested as a chemotaxonomic basis [4,5], but since glucans have linear structures the number of different spectra is limited and a better chemotyping is obtainable with the greater number of possible structures of the mannose-containing polysaccharides.

In the present study, mannose-containing heteropolysaccharides were prepared from 23 different lichens of 13 genera via alkaline extraction, followed in 19 cases by Fehlings precipitation, and then precipitation with Cetavlon at pH 8.5 in the presence of borax. In 4 others, where the extracted heteropolymer contained uronic acid and precipitation would occur at pH 7.0 as well, only a Fehlings purification was carried out. The C-1 regions of the ^{13}C NMR spectra, as previously observed in a brief examination of 8 lichen species, were typical of the lichen and their shifts were often typical of chemical structures suggesting a rapid method for chemotyping lichens [6]. The spectra are now arranged into 5 Groups, based on principal signals in common (Tables 1 and 2) which indicate possible structural similarities, some of which have been confirmed by further chemical analysis [6]. Intrageneric and intrafamily similarities of chemotypes

* Corresponding author.

SSDI 0008-6215(94)00257-6

were more frequent than intergeneric and interfamily ones, but marked differences were sometimes observed within the same genus and family.

Group A.—Similarities occur between C-1 regions of ^{13}C NMR spectra of heteropolysaccharides obtained from the *Parmotrema* spp. (family, Parmeliaceae): *P. cetratum* (Fig. 1A), *P. sulcata,* and *P. araucaria* have signals in common at 99.5, 100.8 (small), 101.8, 102.9, and 104.6 ppm. *P. sulcata* [7] and *P. cetratum* [8] are known to contain (1 → 6)-linked Man*p* main-chain units that are mainly unsubstituted and disubstituted at 0-2 and 0-4 with α-Gal*p* and β-Gal*p* side-chains, respectively. As noted previously [7], the spectrum of the polysaccharide of *P. sulcata* is similar to that of *Cetraria islandica* [9] and it is now clear that there are signals in common with those of *Usnea* sp. [7] and *Evernia prunastri*, both of the family Usneaceae.

Group B.—The C-1 region of the polysaccharide of *Stereocaulon paschale* (family Stereocaulaceae) contained prominent signals at 103.7 and 104.9 ppm (Fig. 1B) arising from α-Man*p*-(1 → 2) and β-Gal*p*-(1 → 4) side-chains which are respective substituents of (1 → 6)-linked α-Man*p* main chains [7]. These signals were also present in spectra of heteropolysaccharides from *Cladonia alpestris* (family, Cladoniaceae) [10] and *C. confusa* [10] having similar chemical structures and in that of *Pseudocyphellaria aurata* (family, Stictacea).

Group C.—The C-1 region of the polysaccharide of *Cladonia amaurocraea*, with signals at 102.1 and 104.7 ppm (Fig. 1C) was different from those of Group B and resembled more that of *Sticta* sp. (family, Stictaceae). Although the signals are similar, chemical analyses show some differences. In the heteropolymer of *Sticta* sp., the signals arose from side-chain units of α-Gal*p*-(1 → 2)-α-Man*p* and β-Glc*p* linked (1 → 2) and (1 → 4), respectively, to main chains of (1 → 6)-linked α-Man*p* units [11,12]. In *C. amaurocraea,* nonreducing end-units of Man*p* and Gal*p* are present, but partial acetolysis has not yet been carried out to form oligosaccharides and the exact nature of the side chains is not known [10]. Another difference of *C. amaurocraea* from other *Cladonia* spp. is that it forms a (1 → 6)-linked β-glucan [10], but not nigeran [13].

Group D.—Several intra- and inter-generic resemblances were noted with polysaccharides having C-1 signals at 101.9 and 104.7 ppm. The lichens involved are *Peltigera aphthosa* [7] (family, Peltigeraceae; Fig. 1D), *Ramalina usnea* [9], and *R. ecklonii* (now *celastri*) [14] whose spectra have many similarities, and *Stereocaulon ramulosum* [15]. These signals arise from a (1 → 6)-linked α-Man*p* main chain partly substituted at 0-4 with β-Gal*p* units.

Group E.—A close resemblance exists between the C-1 region of polysaccharides of lichens of the family Umbilicariaceae, namely those of *Actinogyra muehlenbergii* [9] (Fig. 1E), *Umbilicaria pustulata*, and *U. spodochroa* [16], even though those of the latter genus were prepared in a different way. Common signals are found at 99.9, 101.1, 103.9, and 109.6 ppm (*Actinogyra* Schol. is considered to be synonymous with *Umbilicaria* Hoffm. [17]). In the case of the *A. muehlenbergii* structure, the polysaccharide has a (1 → 6)-linked α-D-Man*p* main chain and the larger signals arise from β-Gal*f* units (109.6 ppm) which are substituents at 0-4 of residues also substituted at 0-2 by α-Man*p* side chains (103.9 ppm). The latter are also monosubstituents at 0-2, giving rise to the same signal. In the main chain, 2-*O*-substituted units are responsible for the C-1 signal at 99.9 ppm [7].

Table 1
Chemical shifts of signals in the C-1 region of ^{13}C NMR spectra of mannose-containing polysaccharides of lichens

Lichen	C-1 Signal (ppm)		
	Group A		
Parmotrema sulcata	104.6 (6.7)[a]	104.3 (4.8)	102.9 (10)
	101.8 (7.4)	100.7 (5.2)	99.5 (6.9)
	98.6 (0.8)	95.7 (0.9)	
Parmotrema cetratum	104.6 (7.8)	104.5 (3.9)	102.9 (10)
	101.8 (7.4)	100.7 (3.1)	99.5 (5.2)
Parmotrema araucaria	178.2 (0.8)	105.9 (0.8)	104.6 (7.2)
	104.2 (3.2)	102.8 (10)	101.8 (5.9)
	100.8 (2.9)	100.5 (5.5)	99.5 (0.4)
	96.6 (1.0)	95.7 (0.9)	
Cetraria islandica	109.5 (1.8)	108.5 (0.3)	104.6 (10)
	102.9 (9.2)	101.9 (7.3)	100.9 (2.7)
	99.6 (6.3)	96.3 (0.3)	95.7 (0.4)
Usnea sp.	178.2 (3.0)	104.6 (6.3)	104.5 (7.0)
	104.3 (8.0)	102.8 (10)	101.8 (5.7)
	100.9 (7.0)	99.5 (5.0)	98.2 (3.0)
	97.8 (2.2)	97.4 (3.0)	96.2 (3.5)
Evernia prunastri	178.2 (1.4)	109.8 (2.0)	104.5 (7.8)
	103.8 (2.6)	102.7 (10)	101.8 (7.0)
	101.0 (2.6)	99.6 (7.8)	98.0 (1.2)
	97.4 (0.8)	96.6 (1.6)	
	Group B		
Cladonia alpestris	109.4 (1.5)	105.5 (2.9)	104.7 (5.9)
	103.6 (10)	102.2 (1.5)	100.7 (1.8)
	99.6 (3.7)		
Cladonia confusa	104.7 (9.0)	103.6 (10)	102.3 (1.6)
	100.7 (5.1)	99.6 (2.3)	
Stereocaulon paschale	104.7 (10)	103.8 (5.3)	101.8 (3.7)
	100.6 (1.9)	100.4 (3.0)	99.5 (2.3)
Pseudocyphellaria aurata	105.8 (4.4)	104.8 (6.9)	104.5 (6.5)
	103.6 (10)	101.9 (4.0)	100.7 (2.1)
	99.6 (6.7)		
	Group C		
Cladonia amaurocraea	109.5 (2.1)	105.6 (3.9)	104.7 (9.5)
	103.9 (3.7)	103.3 (3.1)	102.1 (10)
	100.9 (1.8)	99.7 (4.0)	99.1 (3.1)
Sticta sp.	104.6 (9.0)	104.0 (3.7)	103.7 (3.9)
	102.1 (10)	100.6 (3.2)	99.5 (3.2)
	Group D		
Peltigera aphthosa	104.7 (10)	103.4 (1.8)	101.9 (4.2)
	100.9 (1.1)	99.4 (0.5)	
Ramalina usnea	106.4 (2.6)	104.7 (10)	101.9 (8.2)
	100.9 (5.8)		
Ramalina celastri	106.4 (1.3)	104.7 (10)	101.9 (7.2)
	100.9 (2.4)		
Stereocaulon ramulosum	104.7 (10)	103.8 (4.1)	103.4 (2.5)
	101.8 (4.3)	100.4 (3.4)	

Table 1 (continued)

Lichen	C-1 Signal (ppm)		
	Group E		
Actinogyra muehlenbergii	109.6 (8.5)	107.5 (0.7)	103.9 (10)
	102.3 (1.2)	101.1 (5.1)	99.9 (3.4)
Umbilicaria pustulata [b]	109.6 (1.8)	108.7 (2.0)	103.9 (10)
	102.2 (1.6)	101.3 (2.4)	100.9 (1.2)
	101.1 (4.0)	99.9 (3.2)	
Umbilicaria spodochroa [b]	109.6 (8.0)	108.7 (3.3)	103.9 (10)
	102.2 (3.7)	101.3 (3.7)	100.9 (1.7)
	101.1 (3.7)	99.9 (4.0)	
	Miscellaneous		
Newropogon aurantiaco-ater	104.6 (2.1)	102.8 (10)	101.0 (7.9)
	99.7 (6.3)	98.0 (8.8)	
Tornabenia intricata	103.6 (10)	101.5 (0.8)	100.0 (5.3)
Cladonia substellata	104.7 (10)	104.1 (7.4)	103.6 (6.0)
	102.0 (5.4)	100.5 (4.3)	99.6 (2.0)
	98.8 (3.4)	97.8 (4.3)	

[a] Relative peak height in parentheses.
[b] Literature values [16] increased by 0.4 ppm.

Miscellaneous polysaccharides.—C-1 regions obtained from polysaccharides of *Newropogon aurantiaco-ater* [18] (family, Usneaceae), *Tornabenia intricata* [19] (family, Physiaceae), *Usnea meridionalis*, and *Cladonia substellata* were complex and different from each other and do not resemble those of any of the above Groups.

Not all of the polysaccharides prepared by Fehlings precipitation are chemically homogeneous. Those from *E. prunastri*, *U. meridionalis*, an *Usnea* sp., and *P.*

Table 2
C-1 chemical shifts for mannose-containing polysaccharides isolated from lichens having (1 → 6)-linked α-D-Man*p* main chains

Side-chain structure	Shift (ppm)[a]
β-D-Gal*f*-(1 → 4)-	109.6 [7]
β-D-Gal*p*-(1 → 4)-	104.7 [15]
β-D-Glc*p*-(1 → 4)-	104.6 [11]
α-D-Man*p*-(1 → 2)-[α-D-Man*p*-(1 → 2)]-$_{(0 \rightarrow n)}$	103.7 [20]
α-D-Gal*p*-(1 → 2)-	102.8 [21]
α-D-Man*p*-(1 → 2)-[α-D-Man*p*-(1 → 2)]-$_{(0 \rightarrow n)}$	102.2 [20]
α-D-Gal*p*-(1 → 2)-[α-D-Man*p*-(1 → 2)]-$_{(0 \rightarrow n)}$	102.2 [11]
α-D-Glc*p*-(1 → 2)-	101.8 [19]
Main-chain structure	
Non-substituted	101.0 [20]
2-*O*-Substituted by α-D-Man*p* or α-D-Gal*p*	99.8 [20]
4-*O*-substituted with β-Gal*p*	101.8 [9]

[a] Accurate to ±0.1 ppm.

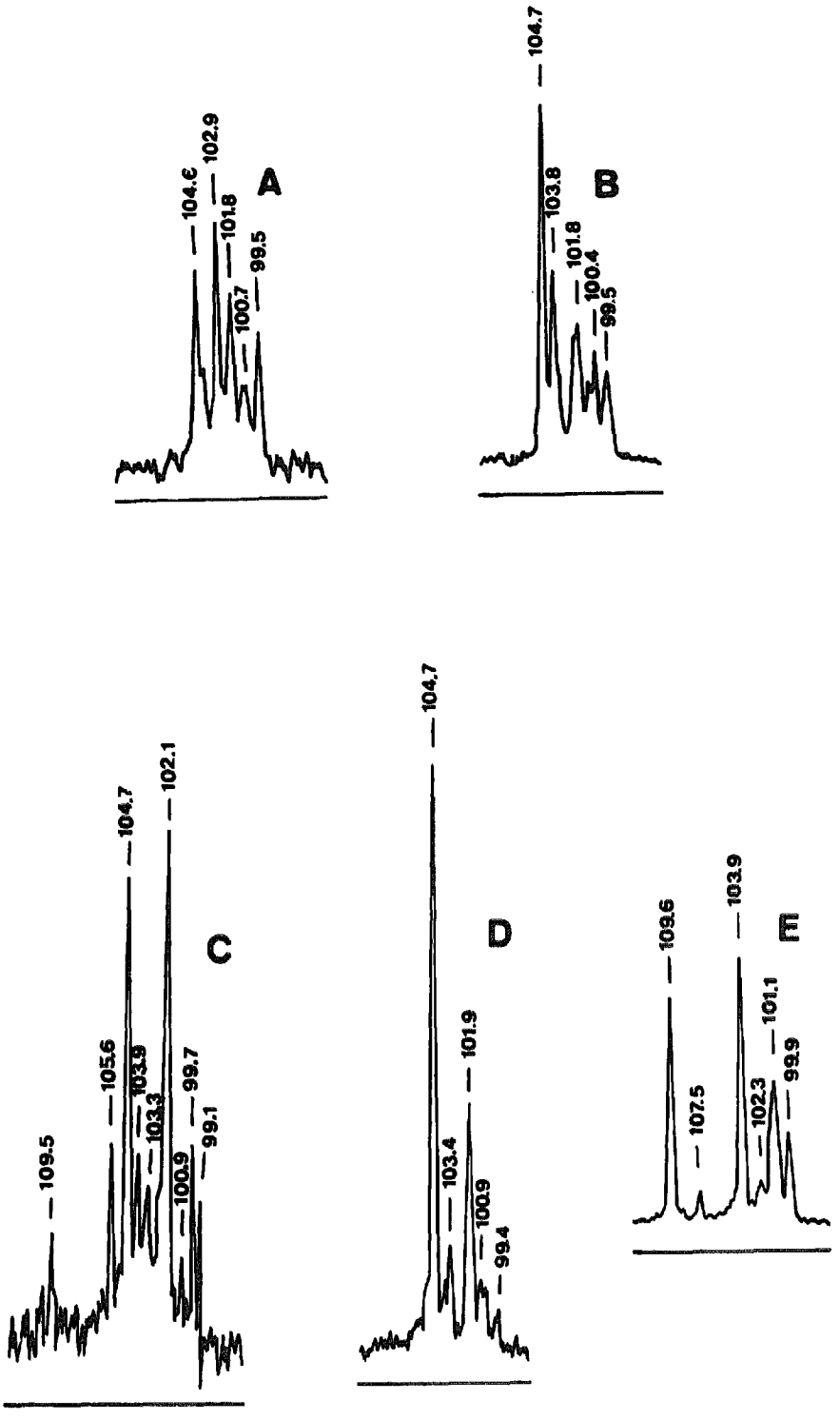

Fig. 1. Representative C-1 regions of ^{13}C NMR spectra of Groups A to E, obtained from mannose-containing polysaccharides of lichens, showing chemical shifts in ppm. Group A: Parmotrema sulcata (A). Group B: Stereocaulon paschale (B). Group C: Cladonia amaurocraea (C). Group D: Peltigera aphthosa (D). Group E: Actinogyra muehlenbergii (E).

araucaria contained neutral and uronic acid-containing components and only the spectra of the Fehlings polysaccharides were examined.

Chemotyping could have two possible applications, namely in correcting the identity of misidentified lichens and in suggesting relationships on the basis of similarities of their mycobionts. DNA homology tests could be used as a final confirmation.

1. Experimental

Extraction of polysaccharide and fractionation with Fehlings solution and Cetavlon.—These procedures were performed as described previously [19].

^{13}C NMR spectroscopy.—Spectra were obtained at 75.6 MHz in D_2O at 33°C. Chemical shifts are expressed in ppm, based on the resonance of tetramethylsilane (0 ppm) determined in a separate experiment and corrected (+0.6 ppm) for the value which would be obtained at 70°C.

Acknowledgments

The authors thank Drs. B. Casu and G. Torri, Istituto G. Ronzoni, Milan, Italy for obtaining the ^{13}C NMR spectra.

References

[1] R. Cooke, *The Biology of Symbiotic Fungi*, Wiley, London, 1977, pp 226–256.
[2] D.L. Hawksworth and D.J. Hill, *The Lichen-Forming Fungi*, Chapman & Hall, New York, 1984, pp. 8–15.
[3] P.A.J. Gorin and J.F.T. Spencer, *Adv. Appl. Microbiol.*, 13 (1970) 25–89.
[4] I. Yokota, S. Shibata and H. Saitô, *Carbohydr. Res.*, 89 (1979) 252–258.
[5] H. Takahashi, T. Kon, I. Yokota and S. Shibata, *Carbohydr. Res.*, 89 (1981) 166–173.
[6] P.A.J. Gorin, M. Baron and M. Iacomini, in M. Galun (Ed.), *CRC Handbook of Lichenology*, Vol III, CRC Press, Boca Raton, FL, 1988, pp 9–23.
[7] P.A.J. Gorin and M. Iacomini, *Carbohydr. Res.*, 142 (1985) 253–267.
[8] M.D.L. Corradi da Silva, P.A.J. Gorin and M. Iacomini, *Phytochemistry*, 34 (1993) 715–717.
[9] P.A.J. Gorin and M. Iacomini, *Carbohydr. Res.*, 128 (1984) 119–132.
[10] M. Iacomini, C.L. Schneider and P.A.J. Gorin, *Carbohydr. Res.*, 142 (1985) 237–251.
[11] M.D.L. Corradi da Silva, M. Iacomini, E. Jablonski, and P.A.J. Gorin, *Phytochemistry*, 33 (1993) 547–552.
[12] P.A.J. Gorin, M. Baron, M.D.L. Corradi da Silva, A.Z.A. Teixeira, and M. Iacomini, *Ciencia e Cultura*, 45 (1993) 27–36.
[13] Y. Nishikawa, K. Ohki, K. Takahashi, G. Kurono, F. Fukuoka and M. Emori, *Chem. Pharm. Bull.*, 22 (1974) 2692–2702.
[14] A.M. Miceno, P.A.J. Gorin and M. Iacomini, *Agric. Biol. Chem.*, 55 (1991) 1391–1392.
[15] M. Baron, P.A.J. Gorin and M. Iacomini, *Agric. Biol. Chem.*, 53 (1989) 1751–1758.
[16] O. Kjølberg and A.L. Kvernheim, *Acta Chem. Scand.*, 43 (1989) 280–285.
[17] E. Dahl and H. Krog, *Macrolichens*, Universitetsforlaget, Oslo, Norway, 1973.
[18] M. Baron, M. Iacomini, E.S. Fanta and P.A.J. Gorin, *Phytochemistry*, 30 (1991) 3125–3126.
[19] A.Z.A. Teixeira, M. Iacomini and P.A.J. Gorin, *Phytochemistry*, 31 (1992) 3467–3470.
[20] P.A.J. Gorin, *Can. J. Chem.*, 51 (1973) 2375–2383.
[21] P.A.J. Gorin and M. Mazurek, *Carbohydr. Res.*, 105 (1982) 283–287.