Carbohydrate Polymers 85 (2011) 349–355

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Carbohydrate epitopes in glycoprotein from the opportunistic fungal pathogen *Scedosporium apiospermum*

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ARTICLE INFO

Article history:
Received 6 December 2010
Received in revised form 4 February 2011
Accepted 21 February 2011
Available online 26 February 2011

Keywords:
Scedosporium apiospermum
Carbohydrate epitopes
Pseudallescheria boydii

ABSTRACT

Hot aqueous extraction of *Scedosporium apiospermum* mycelium provided glycoprotein (HET-PR-Sa) containing 37% protein and Rha, Rib, Ara, Man, Gal, Glc, and $GlcNH_2$ in a 26:14:17:22:10:5:6 molar ratio. HPSEC showed a mixture. HET-PR-Sa, on reductive, alkaline β-elimination at 25 °C, gave nonreducing oligosaccharide epitopes (OLIGO-Sa) from *O*-linked protein and a polymer (HET-PR-Sa-de-O), which was then β-eliminated at 100 °C to give polysaccharide (HET-Sa). The structure of each fraction (methylation, NMR, and ESI-MS analysis) differed from those of a peptidoglycan (PRM-Pb) from mycelium of related, opportunistic pathogen, *Pseudallescheria boydii*. The predominant nonreducing oligosaccharide formed on β-elimination of PRM-Pb was hexasaccharide **3**, whereas those (OLIGO-Sa) from HET-PR-Sa were tetra- **2** and mixed pentasaccharides. Trisaccharide **1** was also identified and is a conserved structure in both fungi. Structural differences confirmed that *S. apiospermum* is not an anamorph of a *P. boydii* teleomorph.

1. Introduction

The opportunistic pathogen *Scedosporium apiospermum*, present worldwide in plant and soil residues, can infect immunocompetent, as well as immunocompromized patients. A related fungus, *Pseudallescheria boydii*, was originally reported to be its anamorph (de Hoog, Guarro, Gené, & Figueras, 2000; Guarro et al., 2002), although a more recent study has indicated that *P. boydii* and *S. apiospermum* are different species. Heterothallism exists in *S. apiospermum*, so that its teleomorph is now defined as *Pseudallescheria apiosperma* (Gilgado, Gené, Cano, & Guarro, 2010).

In the search for structures that could help in diagnosis of pseudallescheriasis, much attention has been paid to the study of *P. boydii* cell-wall antigens.

The importance of surface glycoconjugates of *P. boydii* in cell-cell recognition has been demonstrated (Pinto et al., 2004). Its peptidorhamnomannan (PRM-Pb) was prepared, via extraction of mycelium with hot phosphate buffer, and purified. It lowered adhesion by 50–60% and endocytic indices of conidia and epithelial (HEp) cells and pre-treatment of conidial cells with anti-PRM polyclonal antibody inhibited the interaction in a dose-dependent manner. *O*-Glycosylation is critical for fungal adhesion to host cells, de-*O*-glycosylation of PRM inhibiting adhesion of *P. boydii* conidia to epithelial cells, as well as preventing their endocytosis.

Surface carbohydrates of many pathogens are important for early activation of the innate immune response and their subsequent destruction. For example, Bittencourt et al. (2006) described the participation of a cell-wall (1→4)-linked α-glucan in *P. boydii* internalization by macrophages. It stimulated the secretion of inflammatory cytokine by macrophages and dendritic cells and induced cytokine secretion by cells of the innate immune system with a mechanism involving TLR-2, CD14, and MyD88.

The glycoprotein PRM-Pb from *P. boydii* has a complex carbohydrate structure, shown by methylation analysis (Pinto, Mulloy, Haido, Travassos, & Barreto-Bergter, 2001) and by derived, nonreducing oligosaccharide fragments formed on mild, reductive, alkaline β-elimination of its *O*-linked glycopeptides (Pinto, Gorin, Wait, Mulloy, & Barreto-Bergter, 2005). The carbohydrate epitopes in PRM-Pb are now compared, by detailed structural studies, with those present in an aqueous extract of mycelium of *S. apiospermum* (HET-PR-Sa), to determine the degree of relationship between the two pathogenic fungi.

2. Material and methods

2.1. Microorganism and growth conditions

A culture of *S. apiospermum* was kindly supplied by Dr. J. Guarro, Unitat de Microbiologia, Facultat de Medicina e Institut d'Estudis

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doi:10.1016/j.carbpol.2011.02.033

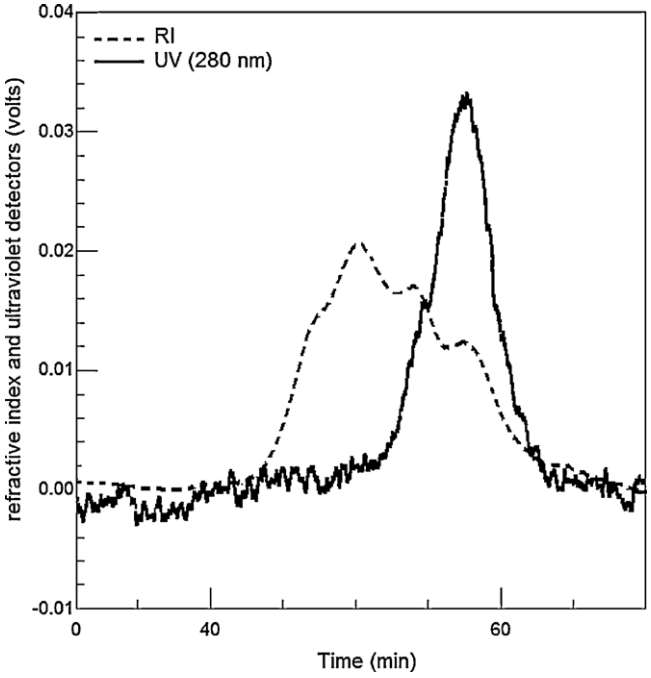

Fig. 1. HPSEC of crude aqueous extract (HET-PR-Sa), using RI and UV detectors.

Avançats, Réus, Spain, as RK107-0417, Clade 5. It was maintained on (g L^{-1}): peptone, 10; yeast extract, 5; glucose, 40; agar, 0. Cells were grown on Sabouraud solid slants, and then inoculated into Erlenmeyer flasks (500 mL) containing culture medium (200 mL) and incubated at 25 °C in Sabouraud liquid medium for 7 days with shaking. Cultures were then transferred to the same medium (3 L) and incubated at 25 °C for 7 days with shaking, the mycelium filtered off, washed with distilled H_2O, and stored at 0 °C (yield wet weight ~135 g).

2.2. Extraction of *S. apiospermum* mycelium to give crude glycoprotein HET-PR-Sa: characterization of derived products

The crude glycoprotein was extracted from *S. apiospermum* mycelium using 0.5 M phosphate buffer, pH 7.2, at 100 °C for 2 h. The filtrate was then dialyzed, evaporated to a small volume, and freeze-dried (yield 1.30 g).

2.2.1. Analytical methods

The methods used for analysis of HET-PR-Sa and derived fractions (Fig. 1), were as described for analysis of the glycoconjugate from *Scedosporium prolificans* (Barreto-Bergter et al., 2008). They were: (1) determination of carbohydrate, protein, and hexosamine contents, (2) HPSEC analysis, carried out with the size-exclusion equipment of Waters, to determine M_w values, using an refractive index detector (a UV absorption detector was now used to detect protein), (3) characterization and quantification of monosaccharide components, via hydrolysis, followed by conversion to alditol acetates and GC-MS examination, (4) methylation analysis: preparation of per-*O*-methylated derivatives, followed by conversion to partly *O*-methylalditol acetates and GC-MS, (5) conventional NMR examination, carried out according to the Bruker manual, with HSQC spectra being obtained of samples insufficient for direct ^{13}C NMR analysis, and (6) ESI-MS^1 and ESI-MS^2 on samples in 0.001 M lithium chloride, to give ions of Li^+ adducts, avoiding uncertainty in structural assignments, since Na^+ and K^+ adducts are normally detected. For fractionation of oligosaccharide mixtures formed on β-elimination, both Biogel P-2 column chromatography and HPLC-RID-MS were used (see Section 2.4).

2.3. Analysis of crude extract HET-PR-Sa and derived fractions

Following the method of Yen and Ballou (1974), HET-PR-Sa (263 mg) was added to a stirred solution of 0.1 M NaOH/0.5 M $NaBH_4$ (5 mL). After 60 h at 25 °C, it was added to aq. HOAc, followed by dilution and addition of mixed Amberlite IR-120 (H^+ form) and Dowex 1-X8 (OAc^- form), which were filtered off and the filtrate evaporated to dryness. The residue was subjected to repeated dissolution in MeOH (3×), then evaporation, to remove boric acid as trimethyl borate. The product was dialyzed, using a SPECTRUM Spectra/Por membrane MICRO: 12–14,000, to give retained glycoprotein (HET-PR-Sa-de-O, 139 mg) and eluted β-eliminated oligosaccharides (OLIGO-Sa, 73 mg).

To a portion (5.0 mg) of the eluted fraction, was added an allitol internal standard, the mixture then being acetylated, and free mannitol and glucitol components identified and quantified (GC–MS).

A sub-milligram portion of the β-eliminated oligosaccharide fraction was examined by ESI-MS^1 and ESI-MS^2.

The β-eliminated polymer (HET-PR-Sa-de-O, 103 mg) was similarly treated with aqueous NaOH–$NaBH_4$, but at 100 °C for 4 h. Retained on dialysis was polysaccharide HET-Sa (25 mg), which was protein-free. Dialyzed material was acetylated with Ac_2O-pyridine, and the product de-*O*-acetylated in methanolic NaOMe, the solution then being neutralized and deionized to give a trace of low-molecular weight product (UP-Sa; 4.7 mg).

2.4. Fractionation of β-eliminated oligosaccharides

2.4.1. Biogel P2 column fractionation of oligosaccharide mixture

The β-eliminated mixture (OLIGO-Sa) was applied to a Biogel P2 column (140 × 2.8 cm i.d., v_o 275 mL), eluted at 0.9 mL min^{-1} to give fractions of 4.5 mL (Barreto-Bergter et al., 2008). The carbohydrate content of each was determined, using phenol-H_2SO_4 colorimetry (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Twelve fractions were obtained and each was submitted to ESI-MS (Li^+ adducts). Only fractions 8 (5.4 mg, *m*/*z* 659) and 12 (1.3 mg, *m*/*z* 351) were pure enough for further analysis.

2.4.2. Oligosaccharide fractionation by HPLC-RID-MS

The mixture of β-eliminated oligosaccharides was also fractionated by high performance liquid chromatography (HPLC–Shimadzu, LC-10A model). Employed was a 200 mm × 10 mm Rezex RSO-Oligosaccharide column (Phenomenex) with a particle size of 12 μm. An isocratic elution was carried out with H_2O at 0.3 mL min^{-1} and a column oven was at 75 °C. Samples were treated with Amberlite IR120 (H^+ form) to remove cations. Aided by a syringe pump at 10 μL min^{-1} and "T" joint, a solution of 0.01 M lithium chloride was post-run co-injected with column eluant into an ESI-MS source to provide positive detection $(M + Li)^+$ (Fig. 4H). After being identified, the oligosaccharide components were fractionated using HPLC-RID with collection of 4 mL fractions. To this, the oligosaccharide mixture was prepared in water at 10 mg mL^{-1}, with each injection volume of 0.25 mL. The fractions of interest were combined and freeze-dried, to give tri- (0.3 mg), tetra- (1.5 mg), and pentasaccharide fractions (1.0 mg).

3. Results

3.1. Preliminary structural examination of crude HET-PR-Sa

The polymeric extract HET-PR-Sa, obtained by hot phosphate buffer extraction of *S. apiospermum* mycelium, contained 37% protein and Rha, Rib, Ara, Man, Gal, Glc, and $GlcNH_2$ in

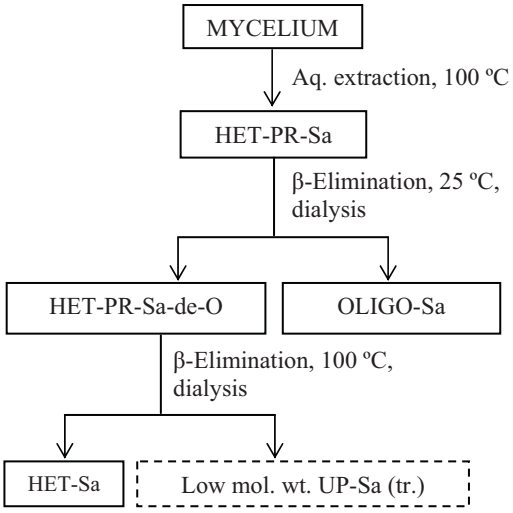

Fig. 2. Flowchart for fractions obtained from the crude aqueous extract of *S. apiospermum* mycelium.

a 26:14:17:22:10:5:6 molar ratio. HPSEC, using an RI detector, showed it to contain 4 components with M_w centered at 1.335×10^5. Only that with the smallest molecular mass was protein-positive, as shown by UV detection (Fig. 1).

3.2. Processing of crude HET-PR-Sa

The extract HET-PR-Sa (see flowchart, Fig. 2), was treated with aq. NaOH–$NaBH_4$ at 25 °C to yield polymeric HET-PR-Sa-de-O, and a mixture of nonreducing oligosaccharides (OLIGO-Sa). The polymer was similarly treated, but at 100 °C, to give polysaccharide (HET-Sa) and a minor, low molecular weight fraction (UP-Sa).

3.2.1. Structural analysis of HET-PR-Sa

Methylation analysis (Table 1) of HET-PR-Sa gave rise to partially *O*-methyated alditol acetates, corresponding principally to nonreducing end-units of Rha*p* (11%), Man*p* (12%), Gal*p* (15%), and Glc*p* (17%), with 2-*O*- (15%), 3-*O*- (10%) and 6-*O*-substituted Man*p* (4%), and 2-*O*-substituted Ara*f* units (7%).

HET-PR-Sa gave rise to a complex ^{13}C NMR spectrum with at least 13 C-1 signals (Fig. 3A). The complete spectrum (not shown) also contained signals typical of protein. Its H-6/H-5 COSY region contained 4 Rha*p* signals (Fig. 3B).

3.2.2. Components of β-eliminated polymer HET-PR-Sa-de-O

The polymer contained 33% protein and Rha, Ara, Man, Gal, Glc, and $GlcNH_2$ in a molar ratio of 19:20:25:20:8:8.

3.2.3. Analysis of β-eliminated oligosaccharide mixture (OLIGO-Sa)

OLIGO-Sa, obtained from HET-PR-Sa, contained free mannitol (1.3%) and glucitol (0.1%), as determined by GC-MS of derived acetates. These arose from single *O*-linked units of mannose and glucose respectively.

On acid hydrolysis, OLIGO-Sa gave rise to Rha, Man, Gal, and Glc in a 15:53:18:14 molar ratio. Methylation analysis (Table 1) gave rise to partially *O*-methylated alditol acetates of >5%, corresponding to nonreducing end-units of Rha*p* (17%), Gal*p* (7%), and Glc*p* (18%), 2-*O*- (5%) and 3-*O*-substitiuted Man*p* (14%), and 2-*O*-substituted Man-ol units (15%). GC–MS of a 2,3,4,6-Me_4Man-ol acetate fragment (18%) gave ions, via C-1/C-2 cleavage, having *m*/*z* 117 and 118 with a slight predominance of the former. These arose from 2,6-di-*O*-substituted Man-ol and nonreducing Man*p* units, respectively.

The anomeric portion of the ^{13}C NMR spectrum of the oligosaccharide mixture OLIGO-Sa (Fig. 3D) was complex, containing at least 14 signals.

ESI-MS^1 examination of the oligosaccharide mixture also showed many components (Fig. 4A), represented as Li^+ adducts. Addition to 0.001 M LiCl gave rise to Li^+ adducts, as shown by a mannitol ion with *m*/*z* 189 and not one at *m*/*z* 205 from an Na^+ adduct. Lithiated molecular ions had *m*/*z* 821 ($RhaHex_3$Hex-ol), 805 (Rha_2Hex_2Hex-ol), 659 ($RhaHex_2$Hex-ol), 643 (Rha_2HexHex-ol), 513 (Hex_2Hex-ol), 497 (RhaHexHex-ol), 351 (HexHex-ol), and 189 (Hex-ol). In three cases, ESI-MS^2 fragments were formed via elimination of Rha and Hex units from branched structures. The pentasaccharide ion with *m*/*z* 821 formed fragments with *m*/*z* 675 and 659, by removal of Rha and Hex terminal units (Fig. 4B), as did another pentasaccharide having *m*/*z* 805 (Fig. 4C), and a tetrasaccharide with *m*/*z* 659 (Fig. 4D). Linear structures were shown by

Table 1
Comparison of partially *O*-methylated alditol acetates and structural components, determined by methylation-GC–MS analysis of PRM-Pb from *P. boydii* and glycoprotein mixture (HET-PR-Sa) from *S. apiospermum*. Structures of oligosaccharide mixture (OLIGO-Sa), derived from HET-PR-Sa by mild β-elimination. Structure of polysaccharide (HET-Sa) obtained by strong β-elimination.

OMe alditol acetate	Structure	Mol% content			
		PRM-Pb	HET-PR-Sa	HET-Sa	OLIGO-Sa
1,2,3,4,6-Me_5Man	→2)-Man-ol	–	–	–	15
1,2,3,4,5-Me_5Man	→6)-Man-ol	–	–	–	–
2,3,4-Me_3Rha	Rha*p*-(1→	12	11	8	17
3,5-Me_2Ara	→2)-Ara*f*		7	4	–
3,4-Me_3Rha	→2)-Rha*p*-(1→	–	1	–	1
2,4-Me_2Rha	→3)-Rha*p*-(1→	13	1	1	–
2,3,4,6-Me_4Man	Man*p*-(1→	2	12	14	18[a]
2,3,4,6-Me_4Gal	Gal*p*-(1→	–	15	18	7
2,3,4,6-Me_4Glc	Glc*p*-(1→	14	17	–	18
3,4,6-Me_3Man	→2)-Man*p*-(1→	17	15	12	5
2,4,6-Me_3Man	→3)-Man*p*-(1→	10.5	10	5	14
2,4,6-Me_3Glc	→3)-Glc*p*-(1→	1	–	–	–
2,3,4-Me_3Man	→6)-Man*p*-(1→	2	4	2	–
2,3,6-Me_3Gal	→4)-Gal*p*-(1→	7.5	–	–	–
2,3,6-Me_3Glc	→4)-Glc*p*-(1→	1	–	4	2
2,6-Me_2Man	→3,4)-Man*p*(1→	–	2	9	3
3,6-Me_2Man	→2,4)-Man*p*-(1→	–	–	–	–
3,4-Me_2Man	→2,6)-Man*p*-(1→	2	5	–	–
2,4-Me_2Man	→3,6)-Man*p*-(1→	14	–	23	–

[a] This derivative arose from nonreducing Man*p* and 2,6-di-*O*-substituted Man-ol units as monodeuterated and nondeuterated C-1 derivatives, respectively.

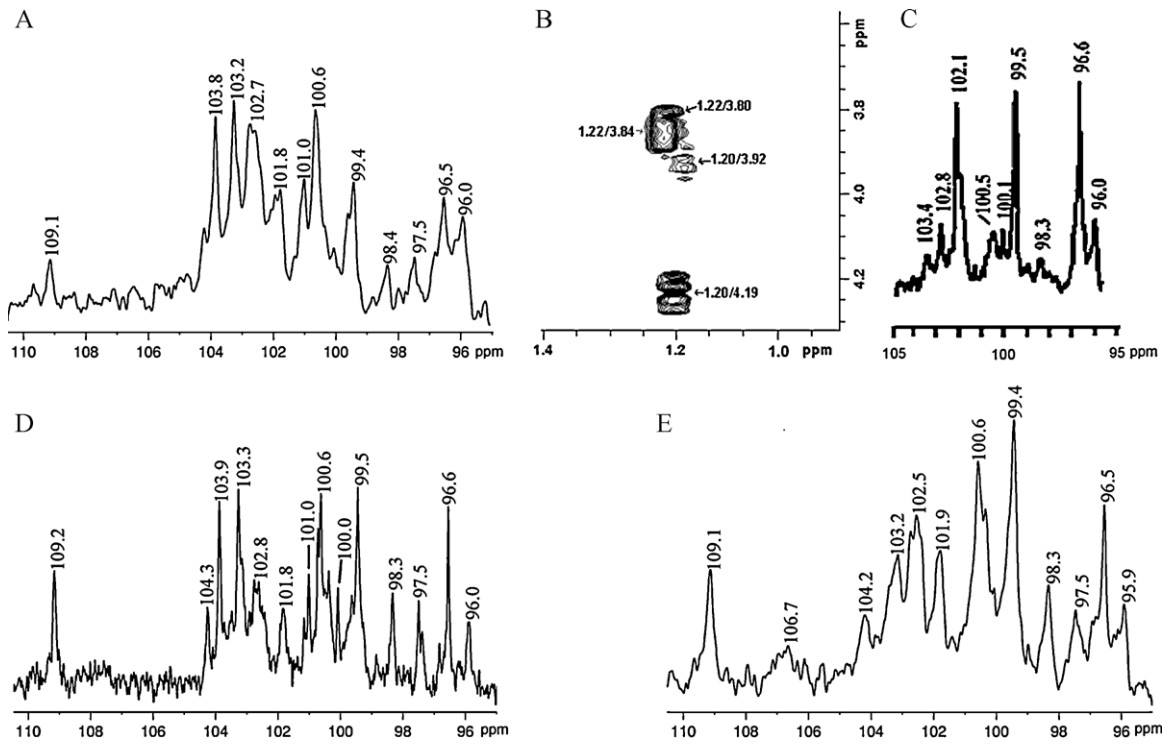

Fig. 3. C-1 region of ^{13}C NMR spectrum of HET-PR-Sa (A), H6/H5 COSY correlation of HET-PR-Sa (B); C-1 regions of *P. boydii* PRM-Pb (C), OLIGO-Sa (D), and HET-Sa (E).

ESI-MS^2 of the RhaHexHex-ol ion with *m*/*z* 497 (Fig. 4E), although the ion with *m*/*z* 513 (Fig. 4F) could have arisen from linear and/or branched Hex_2Hex-ol. The oligosaccharide mixture (OLIGO-Sa) was fractionated on HPLC and Biogel P-2 columns. HPLC provided pure tri-, tetra-, and pentasaccharides (Fig. 4H), but Biogel P-2 column chromatography gave rise only to pure di- and tetrasaccharides. In terms of quantification of oligosaccharide components, HPLC is preferred.

The disaccharide fraction gave a lithiated molecular ion with *m*/*z* 351, arising from HexHex-ol. As with a similar ion, present in that of the β-eliminated mixture (Fig. 4A), it gave an ESI-MS^2 fragment with *m*/*z* 189 by removal of the Hex unit (see Fig. 4G). Methylation analysis showed a Man*p*-(1→2)-Man-ol structure. Its HMQC spectrum contained an anomeric $^1H/^{13}C$ signal at δ 5.458/102.0 (Fig. 5A), and two at δ 4.225/80.6 > 4.260/80.6 from H2/C-2 (Fig. 5B). These shifts were identical with those of a standard, now prepared by $NaBH_4$ reduction of α-Man*p*-(1→2)-Man (Gorin & Perlin, 1956).

The trisaccharide, obtained from the HPLC column, gave rise to, a molecular ion with *m*/*z* 497 (RhaHexHex-ol), and others on ESI-MS^2 with *m*/*z* 351 and 189, corresponding to sequential removal of Rha and Hex units (Fig. 4E). Its HSQC spectrum (Fig. 6C) contained anomeric $^{13}C/^1H$ signals with δ 97.0/4.91 and 101.2/5.00 from α-Rha*p* and α-Man*p* units, respectively. 3-*O*-Substituted α-Man*p* and 2-*O*-substituted Man-ol signals were present at δ 79.6 and 80.1, respectively (Fig. 6D). Methylation analysis gave rise to *O*-methylalditol fragments, which showed nonreducing end-units of Rha*p* and 2-*O*-substituted mannitol and an internal unit of 3-*O*-substituted Man*p*, the overall data indicating an α-Rha*p*-(1→3)-α-Man*p*-(1→2)-Man-ol structure (**1**).

The tetrasaccharide **2**, obtained from the Biogel P-2 column, provided a lithiated molecular ion, as in Fig. 4D, with *m*/*z* 659 ($RhaHex_2$Hex-ol). Similarities in shifts of ^{13}C NMR (Fig. 6A) and HSQC spectra (Fig. 6B) indicated that trisaccharide **1** was a building block in **2**. Methylation analysis of the tetrasaccharide provided partially *O*-methylated alditol acetates of monodeuterated 2,3,4-Me_3Rha, nondeuterated 2,3,4,6-Me_4Man (= 1,3,4,5-Me_4Man) from 2,6-di-*O*-substituted Man-ol, and monodeuterated 2,3,4,6-Me_4Gal and 2,4,6-Me_3Man in a 1.0:0.75:1.1:1.1 molar ratio, consistent with structure **2**. Its NMR spectrum

97.0 101.2, 79.6 80.1
α-Rha*p*-(1→3)-α-Man*p*-(1→2)-Man-ol
1

96.7 101.1, 80.2
α-Rha*p*-(1→3)-α-Man*p*
1
↓
2 2
β-Gal*p*-(1→6)-Man-ol
103 .2 80.2

contained α-anomeric $^{13}C/^1H$ signals at δ 96.6/4.91 and δ 101.0/5.00 and a β-anomeric signal from Gal*p* units at δ 103.1/4.40 (Fig. 6B).

The pentasaccharide fraction gave molecular ions with *m*/*z* 821 ($RhaHex_3$Man-ol > 805 (Rha_2Hex_2Man-ol), as in Fig. 4A and H, and according to ESI-MS^2, had branched structures with Rha and Hex terminal units (Fig. 4B). The anomeric region of its HSQC spectrum contained 5 signals (Fig. 6C), indicating a mixture. In agreement, methylation analysis indicated terminal units of Glc*p*, Gal*p*, and Rha*p*, with 3-*O*-substituted Man*p* and 2,6-di-*O*-substituted Man-ol units.

3.2.4. Structural components of polysaccharide HET-Sa

HET-Sa, obtained by vigorous β-elimination, contained Ara, Rha, Man, Gal, and Glc in a molar ratio of 22:13:33:24:8. It was free of glucosamine and protein.

The anomeric portion of its ^{13}C NMR spectrum showed its structural complexity, containing at least 12 signals (Fig. 3E).

Methylation analysis (Table 1) provided partially *O*-methylated alditol acetates, corresponding mainly (>5%) to nonreducing end-units of Rha*p* (8%), Man*p* (14%), and Gal*p* (18%), with 2-*O*- (5%), 3-*O*- (14%), 3,4- (9%), and 2,6-di-*O*-substituted Man*p* units (23%).

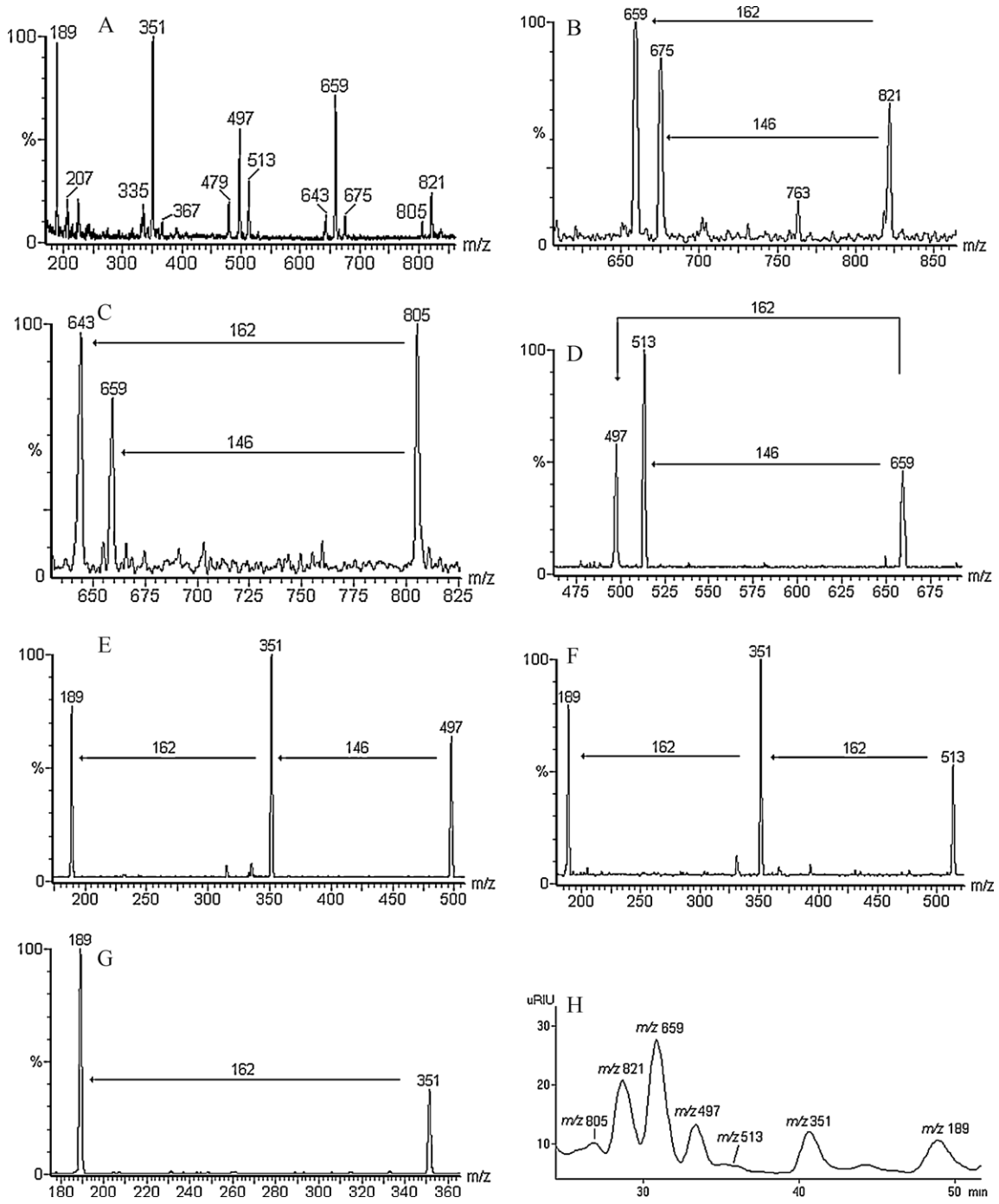

Fig. 4. Lithiated ESI-MS[1] in full-scan mode of β-eliminated oligosaccharide mixture from RMP-Sa (A). ESI-MS[2] fragments arising from *m*/*z* 821 (B), 805 (C), 659 (D), 513 (E), 497 (F), and 351 (G). $\xrightarrow{146}$ and $\xrightarrow{162}$ indicate removal of terminal Rha and Hex units, respectively. HPLC–MS profile of mixture (H).

4. Discussion

Despite the growing importance of *Pseudallescheria*/*Scedosporium* infections, very little is known about the physiology and biochemistry of these neglected tropical pathogens. Characterization of cell-wall glycoconjugates of these fungi should lead to a better understanding of their fungus–host interactions, such as fungal adhesion, internalization, and host immune evasion mechanisms.

Comparison of carbohydrate epitope structures of glycoprotein PRM-Pb, present in mycelium of *P. boydii* (Pinto et al., 2001; Pinto et al., 2005), showed it to contain similar monosaccharides (Rha, Man, Gal, and Glc in a 19.5:64:10:5.5 molar ratio) as those of *S. apiospermum*. However, methylation analysis now showed structural differences. HET-PR-Sa of *S. apiospermum* (Table 1) contained Rha*p* predominatly as nonreducing ends, compared with nonreducing end- and 3-*O*-substituted units in PRM-Pb (Pinto et al., 2001). Furthermore, nonreducing end-units of Gal*p* were present in HET-PR-Sa, but absent in RPM-Pb, whereas 4-*O*-substituted Gal*p* units were present in RMP-Pb, but absent from HET-PR-Sa. Although the anomeric regions of the ^{13}C spectra of the *S. apiospermum* polymeric fractions (Fig. 3A, D, and E) were more complex than that of *P. boydii* (Fig. 3C), there were some signals in common.

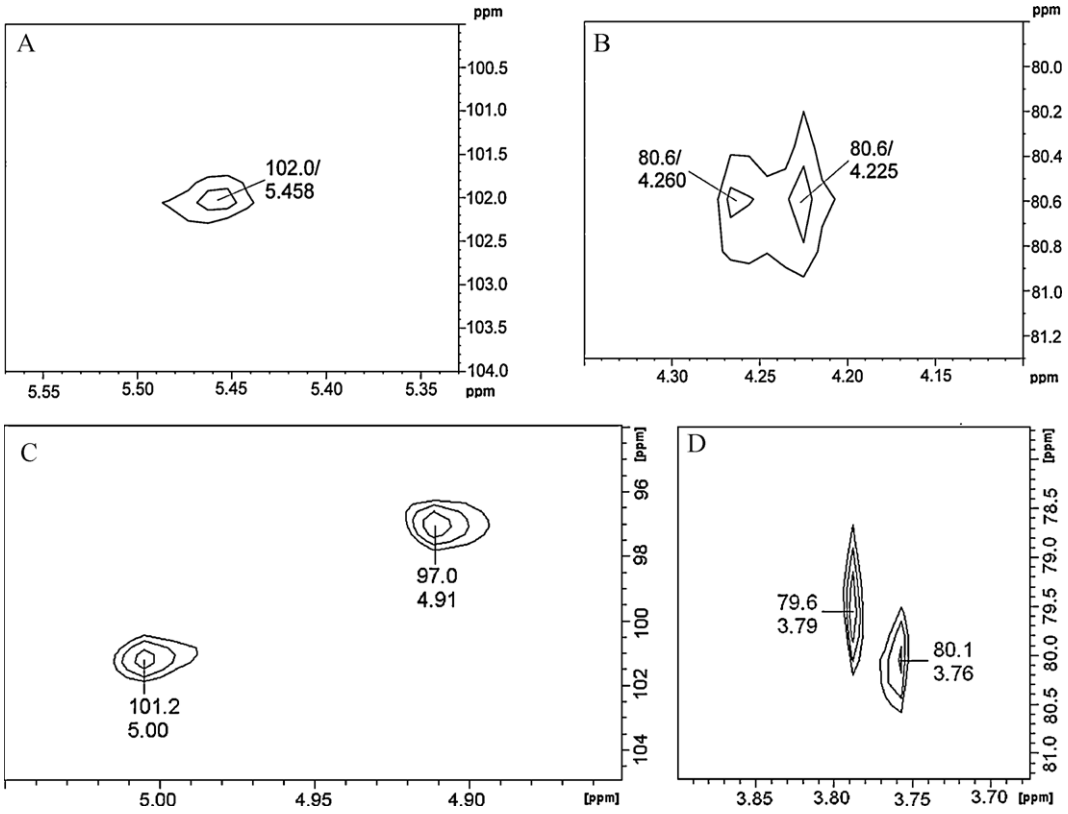

Fig. 5. Selected portions of HMQC spectrum of disaccharide (A and B) from Biogel P-2 and HSQC spectrum of trisaccharide (C and D) by HPLC fractionation.

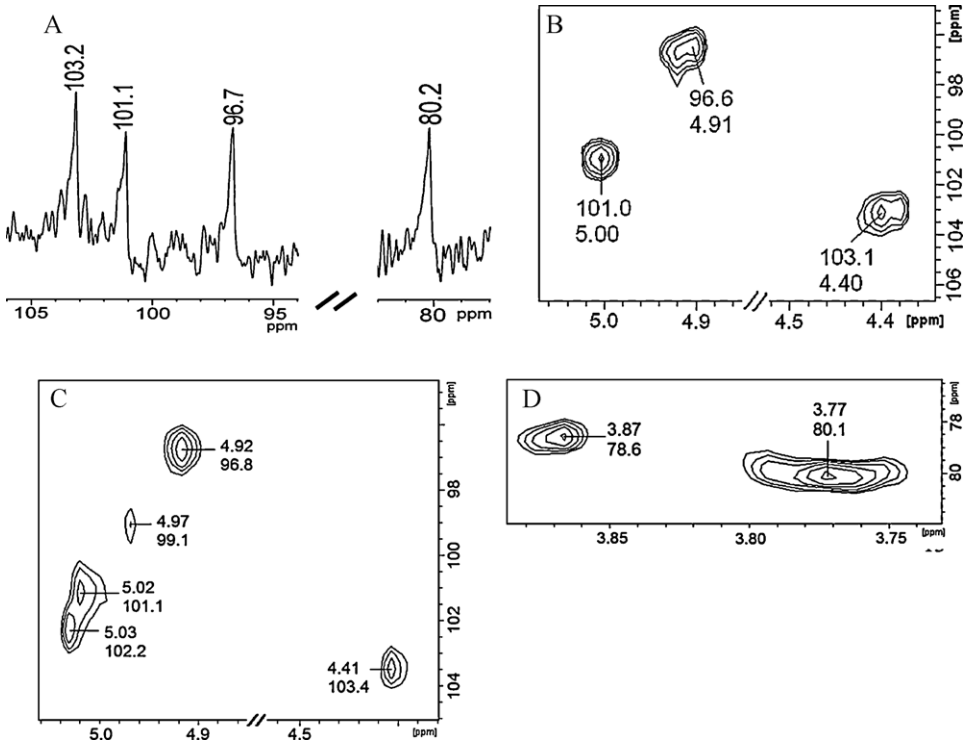

Fig. 6. Selected portions of ^{13}C NMR spectrum of tetrasaccharide fraction from Biogel P-2 (A and B) and HSQC spectrum of pentasaccharide (C and D) from HPLC fractionation.

Mild, reductive β-elimination of HET-PR-Sa gave a mixture of nonreducing oligosaccharides (OLIGO-Sa). Its largest molecular ions were from pentasaccharides $RhaHex_3Man$-ol > Rha_2Hex_2Man-ol (Fig. 4A and H), compared with hexasaccharide **3**

α-Rha*p*-(1→3)-α-Rha*p*-(1→3)-α-Man*p*
1
3 ↓
2
α-Glc*p*-(1→4)-β-Gal*p*-(1→6)-Man-ol

from mycelium of *P. boydii* (Pinto et al., 2005). Although **2** from HET-PR-Sa is a building block of structure **3**, another difference in oligosaccharide structures from *P. boydii* mycelium are the predominance of consecutive Rha*p* units (Pinto et al., 2005), in contrast with those from that of *S. apiospermum*, shown by HPLC-MS to contain only a small peak of an oligosaccharide with *m*/*z* 805 from Rha_2Hex_2Hex-ol. This agreed with methylation data (Table 1), which showed that 2-*O*-substituted Rha*p* units were present as trace components in crude glycoprotein extract HET-PR-Sa and OLIGO-Sa (1%) and 3-*O*-substituted Rha*p* units in HET-PR-Sa and HET-Sa (1%). This compares with 13% of 3-*O*-substituted Rha*p* units in PRM-Pb from *P. boydii* (Pinto et al., 2005).

5. Conclusions

The cell-wall structures of carbohydrates present in glycoproteins, isolated from mycelia of *P. boydii* and *S. apiospermum*, are structurally different. This supports the more recent finding of Gilgado et al. (2010) that they are not respective teleomorph and anamorph of the same species. However, of the many different carbohydrate epitopes present in glycocomplexes of opportunistic, fungal pathogens *P. boydii* (Pinto et al., 2001), *S. prolificans* (Barreto-Bergter et al., 2008), and now *S. apiospermum*, an α-Rha*p*-(1→3)-α-Man*p*-(1→2)-α-Man*p*-(1→ structural component is conserved.

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