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Carbohydrates present in the glycoprotein from conidia of the opportunistic pathogen *Scedosporium prolificans*

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ABSTRACT

Hot aqueous extraction of conidia of *Scedosporium prolificans* gave a heterogeneous glycoprotein (RMP-Sp-Coni) with 41% protein and 2MeRha, Rha, Ara, Man, Gal, Glc, and $GlcNH_2$ in a 2:18:3:47:9:15:6 M ratio, the first report of 2-*O*-methylrhamnose in fungi. Methylation analysis showed nonreducing end- (10%), 2-*O*- (11%), and 3-*O*-substituted Rha*p* (7%), nonreducing end- (8%), 2-*O*- (12%), 3-*O*- (16%), and 2,6-di-*O*-substituted Man*p* (9%), nonreducing end- (4%), 3-*O*- (7%), and 4-*O*-substituted Glc*p* (7%), and nonreducing end-units of Gal*p* (9%). Mild reductive β-elimination of RMP-Sp-Coni cleaved *O*-linked structures to give a mixture of oligosaccharides, of which 2MeRha capping groups were present in $2MeRhaRha_2Hex_2Hex$-ol, $2MeRhaRha_2Hex$-Hex-ol, $2MeRhaRha_2HexHex$-ol, and $2MeRhaRha_2Hex$-ol (ESI-MS–MS). The mixture was fractionated by Biogel P-2 column chromatography and the two predominant isolates were β-D-Gal*p*-(1 → 6)-[2Me-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-D-Man*p*-(1 → 2)]-D-Man-ol, and another lacking the β-Gal*p* unit. Neither was formed from mycelial glycoprotein, although β-D-Gal*p*-(1 → 6)-[α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-D-Man*p*-(1 → 2)]-D-Man-ol was a common component.

1. Introduction

Scedosporium prolificans is a common fungus occurring in soil and plant residues and is an opportunistic pathogen, capable of infecting immunocompetent, as well as immunocompromised patients. Hot aqueous extraction of its mycelium furnished a heterogeneous polymer (RMP-Sp) with 35% protein and 62% carbohydrate. Mild reductive β-elimination provided an oligosaccharide mixture and a resistant polymer, the former consisting mainly of β-D-Gal*p*-(1 → 6)-[α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-D-Man*p*-(1 → 2)]-D-Man-ol, a pentasaccharide lacking β-D-Gal*p* side-chain units, and β-D-Gal*p*-(1 → 6-[α-D-Man*p*-(1 → 2)]-D-Man-ol in a 16:3:1 w/w ratio (Barreto-Bergter et al., 2008). A preliminary report on analysis of a glycoprotein from conidia of *S. prolificans* (RMP-Sp-Coni) described some structural features and the presence of 2-*O*-methylrhamnose residues (Gorin et al., 2008). The analysis is now described in more detail, as well the structures of oligosaccharide epitopes formed on mild reductive β-elimination.

2. Materials and methods

2.1. Microorganism and growth conditions

A culture of *S. prolificans* was supplied by Dr. J. Guarro, Unitat de Microbiologia, Facultat de Medicina e Institut d'Estudis Avançats, Réus, Spain. It was grown in Erlenmeyer flasks containing 200 mL of Sabouraud modified medium (g/L) peptone, 10; yeast extract, 5; glucose, 40; and incubated at room temperature for 7 days with shaking (pre-inoculum). Conidia were grown on Petri plates containing modified Sabouraud medium at room temperature. After 7 days, conidia were obtained by washing the plate surface with phosphate-buffered saline and hyphal fragments and debris were removed by filtration through gauze.

2.2. Extraction of conidia

In a typical experiment, conidia were extracted with 0.05 M phosphate buffer, pH 7.2, at 100 °C for 2 h, and the mixture was then dialyzed. Centrifugation of retained material provided a supernatant, which was evaporated to a small volume and freeze-dried to give crude glycoprotein (541 mg). An aqueous solution was then dialyzed to give retained material (RMP-Sp-Coni, 119 mg).

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2.3. Analytical methods

RMP-Sp-Coni was analyzed using methods employed with the glycoprotein isolated from mycelia of *S. prolificans* (Barreto-Bergter et al., 2008). These were (1) determination of carbohydrate, hexosamine, and protein contents, (2) quantitative and qualitative monosaccharide GC–MS analyses, (3) HPSEC with molar mass (M_w) determination, (4) methylation-GC–MS analyses, and (5) NMR spectroscopy, following conditions described in the Bruker manual, although DEPT was used to enhance –O$\underline{C}$H$_3$ signals and HSQC spectra were obtained with fractions that provided weak ^{13}C NMR spectra, and (6) ESI-MS and ESI-MS–MS of sodiated and lithiated ions, following pre-treatment with traces of NaCl and LiCl, respectively.

2.4. Preparation and fractionation of β-eliminated oligosaccharides on Biogel P-2

According to the method of Yen and Ballou (1974), RMP-Coni (201 mg) was treated with aqueous $NaBH_4$–NaOH at 25 °C for 40 h and following neutralization (HOAc), the solution was treated with Amberlite IR-120 (H^+ form), which was filtered off, and the filtrate freeze-dried. The residue was dissolved in MeOH, and the solution evaporated to remove boric acid. An aqueous solution of the residue was dialyzed through a membrane (Barreto-Bergter et al., 2008). An eluted β-eliminated oligosaccharide mixture (97 mg) was obtained, and was applied to a Biogel P-2 column (140 × 2.8 cm i.d.; v_o 275 mL), which was eluted at 0.9 mL/min to give fractions of 4.5 mL. Thirteen fractions were obtained, ranging in yields from 1.0 to 2.9 mg. Each was assayed colorimetrically for carbohydrate using the phenol–H_2SO_4 reagent (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.5. Controlled Smith degradation of Fractions 1 and 2

Added to each sample (50 μg) in water (2 mL) was added $NaIO_4$ (100 mg), and after 18 h, the solution was treated with a mixture of Amberlite IR-120 (H^+ form) and Amberlite IR-400 (OAc^- form) exchange resins. Filtration and evaporation gave a residue, to which MeOH was added, and the solution was evaporated, a process that removed boric acid and trimethyl borate. The product was partially hydrolyzed in aqueous TFA with pH 2.0 (5 mL) for 30 min at 100 °C (Gorin, Horitsu, & Spencer, 1965). The residue obtained on evaporation was examined by ESI-MS and ESI-MS–MS.

3. Results

3.1. Preliminary analysis of glycoprotein RMP-Sp-Coni

Hot phosphate buffer extraction of conidia provided a crude glycoprotein (RMP-Sp-Coni), which contained 41% protein and 62% carbohydrate with 2MeRha (2-*O*-methylrhamnose), Rha, Ara, Man, Gal, and $GlcNH_2$ in a 2:18:3:47:9:15:6 M ratio. The neutral monosaccharides were analyzed as their derived alditol acetates, which had typical retention times and GC–MS electron impact profiles. Glucosamine was also identified, but quantified colorimetrically.

Unlike the glycoprotein obtained from mycelium (Barreto-Bergter et al., 2008), Cetavlon-borate treatment did not provide a precipitate.

HPSEC, using a refractive index detector, showed RMP-Sp-Coni to be a mixture (Fig. 1) with main components having 18 and 21 kDa.

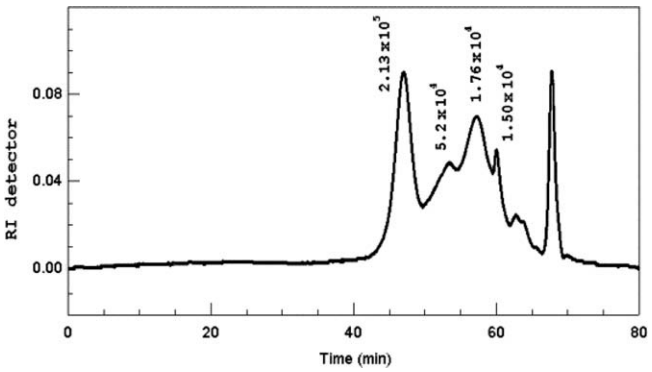

Fig. 1. HPSEC of RMP-Sp-Coni with M_w values.

3.2. Methylation and NMR analysis of RMP-Sp-Coni

Methylation analysis of RMP-Sp-Coni and GC–MS examination of partially *O*-methylated alditol acetates (Table 1) showed a complex structure with nonreducing end- (10%), 2-*O*- (11%), and 3-*O*-substituted Rha*p* (7%), nonreducing end- (8%), 2-*O*- (12%), 3-*O*- (16%) and 2,6-di-*O*-substituted Man*p* (9%), nonreducing end- of Gal*p* (9%), and nonreducing end- (4%), 3-*O*- (7%), and 4-*O*-substituted Glc*p* units (7%). This composition resembled closely the structures present in the extract from mycelia of RMP-Sp (Barreto-Bergter et al., 2008).

RMP-Sp-Coni gave a complex anomeric region in its ^{13}C NMR spectrum (Fig. 2A), with eight main signals ranging from δ 96.5 to 103.6. Its HMQC spectrum (not shown) contained an H-1/C-1 region with a signal at δ 4.43/103.6, arising from a β-pyranosyl structure. All other H-1 signals were from δ 5.10 to 5.46, indicating α-anomers. The ^{13}C spectrum of RMP-Sp-Coni differed from that of the glycoprotein from mycelial RMP-Sp (Fig. 2B) (Barreto-Bergter et al., 2008), but with some signals in common. The structural complexity of RMP-Sp-Coni was shown by its COSY spectrum, which had H-6/H-5 correlations of Rha*p* and 2MeRha units, with signals at δ 1.17/3.61, 1.22/3.70, 1.25/4.03, and 1.27/3.79 (Fig. 2C), arising from four different environments. That of RMP-Sp from mycelium contained three correlations (Barreto-Bergter et al., 2008).

3.3. Analysis of mixture of oligosaccharide epitopes formed on β-elimination of RMP-Sp-Coni

In order to determine structural sequences in RMP-Sp-Coni and some of their structures, it was subjected reductive β-elimination with aqueous $NaBH_4$–NaOH at 25 °C, which liberated carbohydrate *O*-linked to protein, furnishing nonreducing oligosaccharides (Yen & Ballou, 1974). The solution was neutralized (HOAc) and dialyzed, which allowed retention of polymer, probably *N*-linked, and the passage of nonreducing oligosaccharides.

This mixture was shown by ESI-MS to be a complex mixture (Fig. 3A), with main sodiated molecular ions of up to *m/z* 967 (Rha_3-Hex_2Hex-ol) and 981 (2MeRhaRha_2Hex_2Hex-ol), the latter not being formed from mycelia (Fig. 3B; Barreto-Bergter et al., 2008). MS–MS of the *m/z* 967 ion (Fig. 3C) showed removal of *m/z* 146 of Rha and 162 of Hex terminal units from a branched structure. As can be seen, successive fragments showed removal of other units to form a Hex_2Hex-ol core (*m/z* 529). MS–MS of the *m/z* 981 ion indicated removal of a 2MeRha capping group with *m/z* 160 to give a fragment with *m/z* 821 (Fig. 3D). Fig. 3B and C shows sequential removal of the monosaccharide units, starting from the capping groups.

The β-eliminated mixture was treated with cationic exchange resin to remove any compounds containing free amino groups.

Table 1
Partially *O*-methylated alditol acetates and consequent structures formed on methylation analysis of *S. prolificans* glycoproteins from mycelium (RMP-Sp) and conidia (RMP-Sp-Coni), oligosaccharides (RMP-Coni-oligos) formed on β-elimination of RMP-Sp-Coni, and oligosaccharide Fraction 1.

OMe alditol acetate	Structure	Mol% content			
		RMP-Sp[a]	RMP-Sp-Coni	RMP-Sp-Coni-oligos	Fraction 1
1,2,3,4,6-Me_5Man	→2)-Man-ol	–	–	8	2
1,2,3,4,5-Me_5Man	→6)-Man-ol	–	–	10	–
2,3,5-Me_3Ara	Ara*f*-(1→	1	–	–	–
2,3,4-Me_3Rha	Rha*p*-(1→	9	10	7	12
3,5-Me_2Ara	→2)-Ara*f*-(1→	–	–	6	–
2,4-Me_2Rha	→3)-Rha*p*-(1→	7	7	5	10
3,4-Me_3Rha	→2)-Rha*p*-(1→	13	11	9	26
2,3,4,6-Me_4Man	Man*p*-(1→	11	8	∼7[b]	–
	→2,6)-Man-ol	–	–	∼7a[b]	18[c]
2,3,4,6-Me_4Gal	Gal*p*-(1→	13	9	11	17
2,3,4,6-Me_4Glc	Glc*p*-(1→	1	4	10	–
3,4,6-Me_3Man	→2)-Man*p*-(1→	10	12	1	3
2,4,6-Me_3Man	→3)-Man*p*-(1→	14	16	8	19
2,4,6-Me_3Glc	→3)-Glc*p*-(1→	1	7	8	–
2,3,4-Me_3Man	→6)-Man*p*-(1→	4	–	–	–
2,3,6-Me_3Glc	→4)-Glc*p*-(1→	1	7	–	–
3,6-Me_2Man	→2,4)-Man*p*-(1→	1	–	–	–
3,4-Me_2Man	→2,6)-Man*p*-(1→	13	9	–	–
2,4-Me_2Man	→3,6)-Man*p*-(1→	1	–	–	–

[a] Barreto-Bergter et al. (2008).
[b] Deuterated and non-deuterated derivatives (14%) formed on hydrolysis of the *O*-methylated oligosaccharide, $NaBD_4$ reduction, and acetylation sequence. Values of ∼7% each are estimated, based on respective *m/z* 117 and 118 ions of equal intensity.
[c] Only non-deuterated ions were detected.

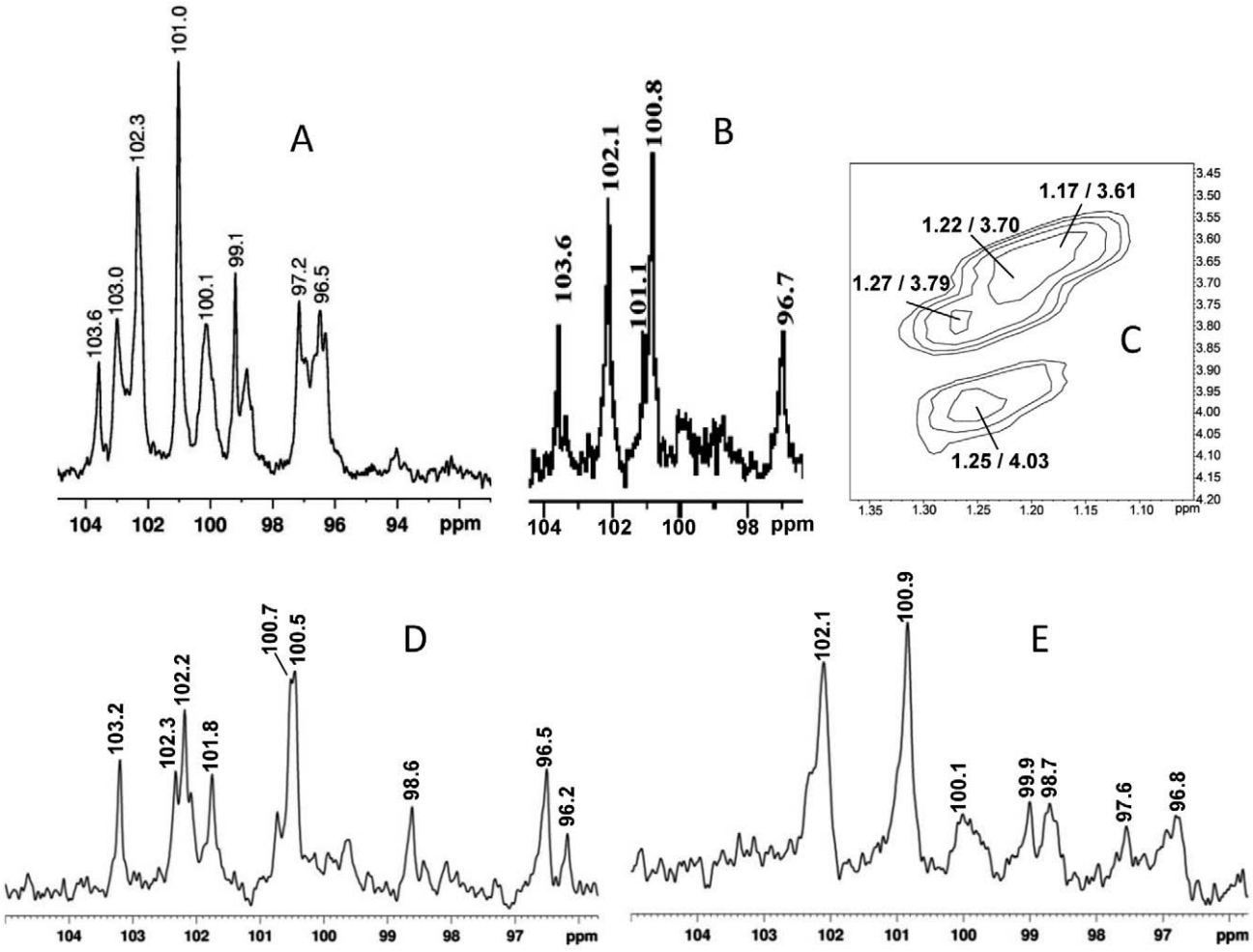

Fig. 2. C-1 region of ^{13}C NMR spectrum of RMP-Sp-Coni (A) and that of RMP-Sp (B) from mycelium. H-5/H-6 correlation of COSY from RMP-Sp-Coni (C), and the C-1 region of mixture of oligosaccharides formed on its mild β-elimination (D). C-1 region of polymer not degraded on β-elimination (E).

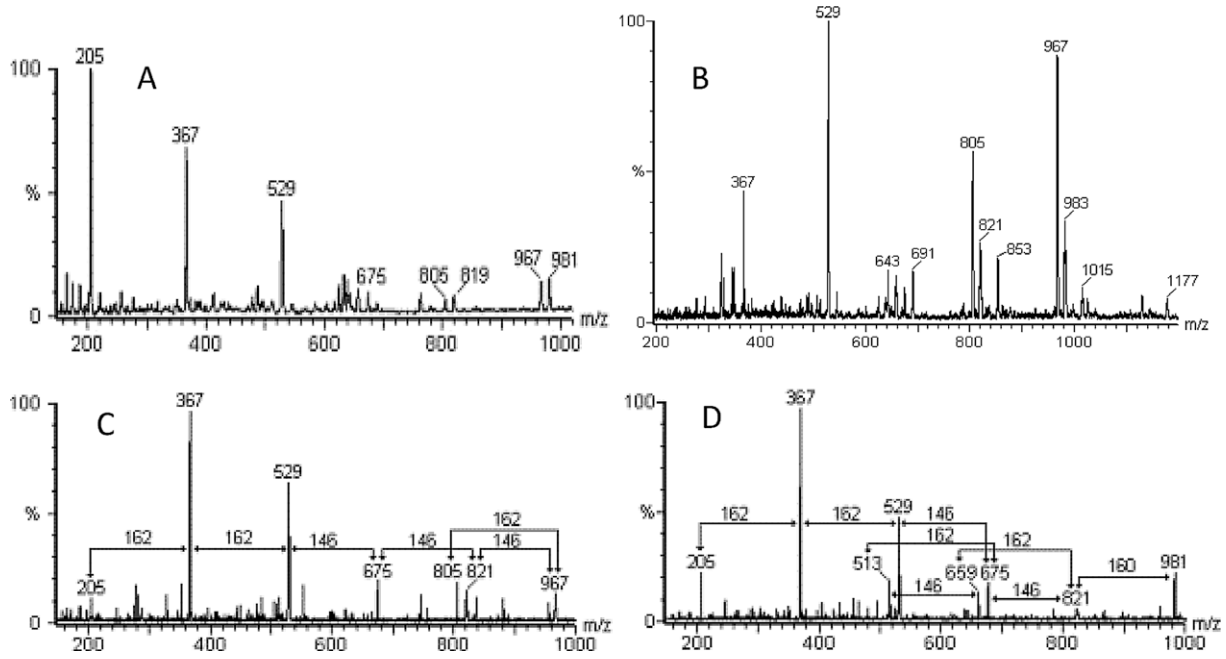

Fig. 3. ESI-MS (Na^+) of nonreducing oligosaccharide mixture, obtained by mild, reductive alkaline β-elimination of RMP-Sp-Coni with ions, *m/z* 981 (2MeRhaRha$_2$Hex$_2$Hex-ol), 967 (Rha$_3$Hex$_2$Hex-ol), 819 (2MeRhaRha$_2$HexHex-ol), 805 (Rha$_3$HexHex-ol), 675 (RhaHex$_2$Hex-ol), 529 (Hex$_2$Hex-ol), 367 (HexHex-ol), and 205 (Hex-ol) (A). ESI-MS of RMP-Sp from mycelium gave ions of Hex$_{6\text{-}1}$Hex-ol, among others (B) [1]. MS–MS of *m/z* 967 from RMP-Sa-Coni gave 821 (Rha$_2$Hex$_2$Hex-ol), 805 (Rha$_3$HexHex-ol), 675 (RhaHex$_2$Hex-ol), 529, 367, and 205 (Hex$_{2\text{-}0}$Hex-ol) (C). MS–MS of *m/z* 981 from RMP-Sp-Coni gave 821 (Rha$_2$Hex$_2$-Hex-ol), 675 (RhaHex-Hex-ol), 659 (Rha$_2$Hex-ol), 529, 367, and 205 (Hex$_{2\text{-}0}$Hex-ol) (D). $\overset{146}{\leftrightarrow}$ denotes MS–MS removal of Rha, $\overset{162}{\leftrightarrow}$ Hex, and $\overset{160}{\leftrightarrow}$ 2MeRha units.

On hydrolysis, 2MeRha, Rha, Ara, Man, Gal, and Glc in a 4:20:5:45:12:14 M ratio (GC–MS of derived alditol acetates).

Methylation-GC–MS analysis of the β-eliminated mixture was carried out, giving partially *O*-methylated alditol acetates. This incorporated a reduction step with $NaBD_4$ (Table 1), which characterized units of 2-*O*- (8%), 6-*O*- (10%), and 2,6-di-*O*-substituted Man-ol. Also present were nonreducing end- (14%), 2-*O*- (1%) and 3-*O*-substituted Man*p* (8%), nonreducing end- (7%), and 2-*O*-substituted Ara*f* (6%), nonreducing end- (9%), 2-*O*- (9%) and 3-*O*-substituted Rha*p* (5%), nonreducing end- (10%) and 3-*O*-substituted Glc*p* (2%), and nonreducing end-units of Gal*p* (11%). MS of the 2,3,4,6-tetra-*O*-methylmannitol acetate fragment gave rise to ions with *m/z* 117 and 118 in the same intensity, which correspond to 2,6-di-*O*-substituted mannitol and nonreducing end-units of Man*p*, respectively. The majority of the *O*-methylalditol acetates fragments were similar to those obtained from RMP-Sp-Coni (Table 1).

The C-1 region of the ^{13}C NMR spectrum of the β-eliminated mixture (Fig. 2D) contained some of the signals obtained from RMP-Sp-Coni (Fig. 2A).

The polysaccharide retained on dialysis contained 2MeRha, Rha, Ara, Man, Gal, and Glc in a 1:8:17:30:4:40 M ratio. The C-1 region of its ^{13}C NMR spectrum contained two main signals at δ 100.9 and 102.1, but lacked a β-Gal*p* signal (Fig. 2E). However, a signal at δ 103.6/4.43 in its HSQC spectrum (not shown) indicated the presence of β-Gal*p* units.

3.4. Pre-examination of β-eliminated oligosaccharide components and their isolation

In order to isolate oligosaccharides from the β-eliminated mixture, it was fractionated on a Biogel P-2 column, as for preparation of oligosaccharides liberated from mycelial glycoprotein (RMP-Sp; Barreto-Bergter et al., 2008). The elution profile of carbohydrate-positive material (Fig. 4), showed a partial resolution. In a preliminary survey, 13 fractions were examined, and microgram samples submitted to ESI-MS, whose molecular ions indicated the presence of 2MeRha units and the number of components.

The lithiated molecular ions of Fraction 1 showed a mixture of three components (Fig. 5A), with *m/z* 965 (2MeRhaRha$_2$Hex$_2$Hex-ol), 951 (Rha$_3$Hex$_2$Hex-ol), and 803 (2MeRhaRha$_2$HexHex-ol). MS–MS of the *m/z* 965 (Fig. 5B) and 803 ions (Fig. 5C) showed removal of *m/z* 160 units giving rise to fragments with *m/z* 805 and 643, respectively, showing removal of 2MeRha capping groups (this showed a less efficient fractionation than was obtained with related oligosaccharides lacking 2MeRha units; Barreto-Bergter et al., 2008).

Fraction 2 was more complex, giving four ESI-MS molecular ions with *m/z* 951 (Rha$_3$Hex$_2$Hex-ol), 803 (2MeRhaRha$_2$HexHex-ol), 789 (Rha$_3$HexHex-ol), and 641 (2MeRhaRha$_2$Hex-ol) (Fig. 5D).

Fraction 7 was even more complex, giving a small *m/z* 673 ion from 2MeRhaHex$_2$Hex-ol (not shown). MS–MS gave fragments showing that 2-*O*-methylrhamnose residues, when present, were the capping groups.

The heterogeneity of these fractions was confirmed by their HSQC spectra (not shown).

3.5. Detailed analysis of components in Biogel P-2 fractions

Fraction 1 contained 2MeRha, Rha, Man, and Gal in a 6:25:48:21 M ratio. Its ^{13}C NMR spectrum had many signals present in those of β-D-Gal*p*-(1 → 6)-[α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-D-Man*p*-(1 → 2)]-D-Man-ol (structure **1**), obtained from mycelium of *S. prolificans* (Fig. 6A; Barreto-Bergter et al., 2008). However, it contained additional signals at δ 98.7 arising from C-1 and δ 79.9 from C-2 of 2-*O*-methylrhamnose residues (Fig. 6B). An –O<u>C</u>H$_3$ signal was not detected, but one appeared at δ 58.2, in its DEPT spectrum (not shown), from β-D-Gal*p*-(1 → 6)-(α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-D-Man*p*-(1 → 2)]-D-Man-ol (structure **2**). Other differences in Fig. 6B, when compared with Fig. 6A, were a smaller signal at δ 101.8, arising from some 2Me-α-Rha*p* rather than α-Rha*p* terminal units. Another smaller signal at δ 103.3 was due to a 2MeRhaRha$_2$HexHex-ol component, which did not

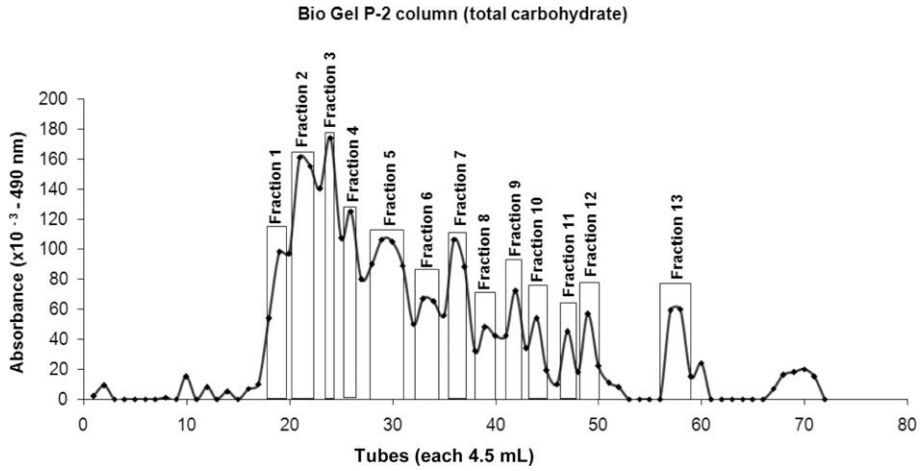

Fig. 4. Elution profile of, on Biogel P-2 column chromatography, β-eliminated carbohydrate mixture.

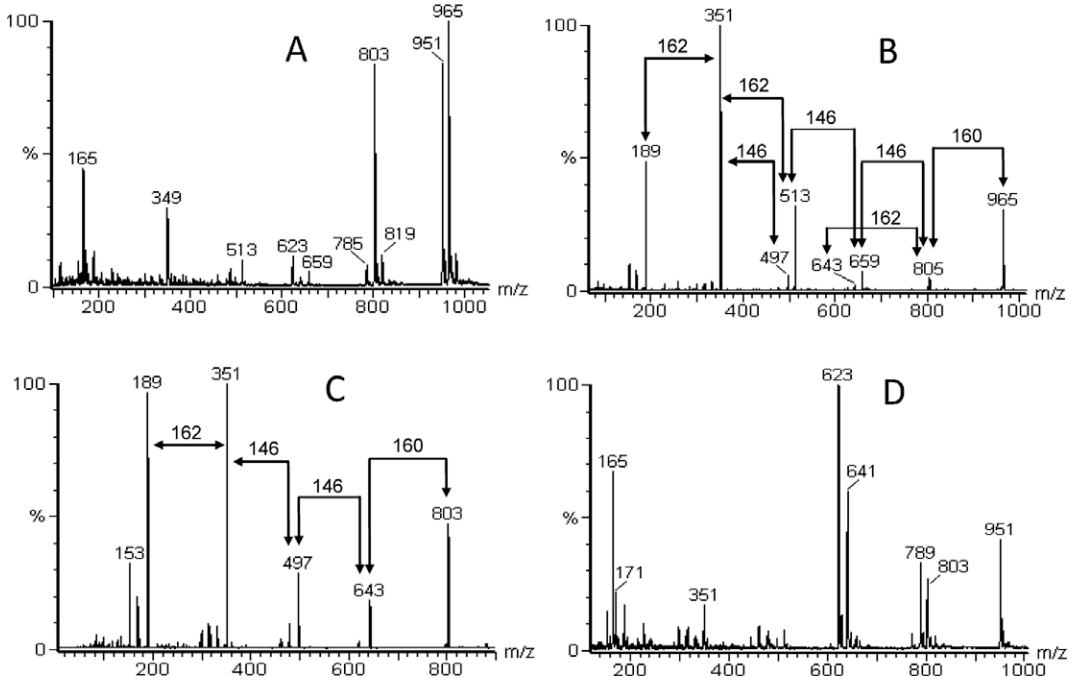

Fig. 5. ESI-MS (Li^+) of Fraction 1 (A). ESI-MS–MS of m/z 965 molecular ions (B) and m/z 803 (C). ESI-MS (Li^+) of molecular ions of Fraction 2 (D).

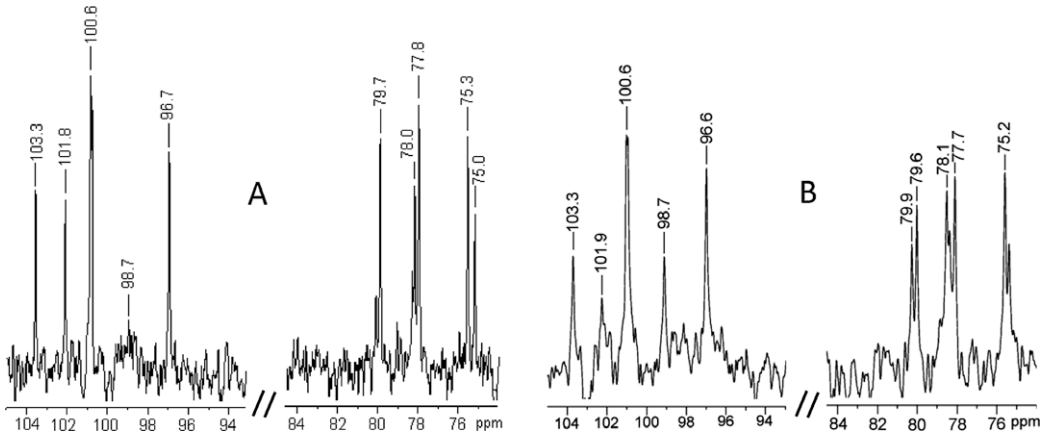

Fig. 6. ^{13}C NMR spectrum of β-eliminated hexasaccharide from glycoprotein of mycelium (A) (Barreto-Bergter et al., 2008) and that of Fraction 1 (B).

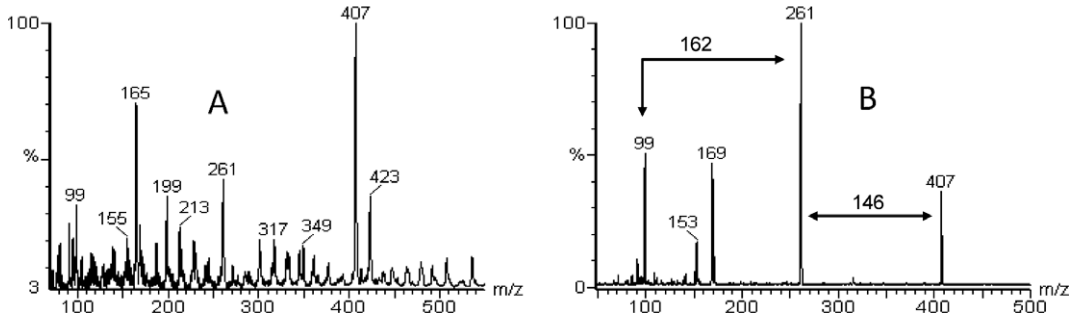

Fig. 7. ESI-MS (A) of lithiated ions of the product formed by controlled Smith degradation of Fraction 1 (A) with molecular ion of *m/z* 407. Its MS–MS fragments (B) had *m/z* 261 (−156 = Rha) and 99 (−308 = RhaMan).

contain a β-Gal*p* side-chain, and corresponded to a molecular ion with *m/z* 830 (Fig. 5A).

Methylation analysis of Fraction 1 (Table 1) provided *O*-methylalditol acetates consistent with the presence of structures **1**, **2**, and 2RhaRha$_2$HexHex-ol.

Further evidence for these structures was provided by a controlled Smith degradation of the fraction, which provided expected Rha-(1 → 3)-Man-(1 ↔ 1)-Glycerol. On ESI-MS, the principal lithiated molecular ion had *m/z* 407 (Fig. 7A), with significant MS–MS fragments having *m/z* 261 (-Rha), 169 (-RhaGlycerol), and 99 (-RhaMan) (Fig. 7B).

Fraction 2 contained 2MeRha, Rha, Man, and Gal in a 4:35:51:10 M ratio. As mentioned above, it gave rise on ESI-MS, to four principal molecular ions from one hexa-, two penta-, and one tetrasaccharide (Fig. 5D). A controlled Smith degradation also gave rise to a product, whose ESI-MS (not shown) had a lithiated molecular ion and MS–MS fragments identical to those of Rha-(1 → 3)-Man-(1 ↔ 1)-Glycerol, derived from Fraction 1. The ^{13}C NMR spectrum of Fraction 2 was weak, but a well-defined HSQC spectrum was obtained (not shown). It contained ^{1}H/^{13}C signals, corresponding to those of ^{13}C obtained from Fraction 1 (Fig. 6B) in the anomeric and *O*-substituted regions, as well as an ^{1}H/^{13}C –OCH$_3$ signal at δ 3.45/58.2.

Fraction 9, on ESI-MS, gave a lithiated molecular ion with *m/z* 513 (Hex$_2$Hex-ol), which on MS–MS gave fragments with *m/z* 351 (-Hex) and 189 (-Hex$_2$). According to its DEPT spectrum (not shown), it was β-D-Gal*p*-(1 → 6)-[α-D-Man*p*-(1 → 2)]-D-Man-ol with signals identical with those of the ^{13}C NMR spectrum of authentic material (Barreto-Bergter et al., 2008). These are C-1 signals at δ 103.3 (β-D-Gal*p*) and 100.8 (α-D-Man*p*), and 79.6 (2-*O*-substituted α-D-Man*p*), and an inverted one at δ 71.0 (6-*O*-substituted D-Man-ol). On methylation analysis, Fraction 9 gave rise to *O*-methylalditol acetates of 2,3,4,6-Me$_4$Gal and 2,3,4,6-Me$_4$Man in 1:2.2 M ratio (GC–MS), the latter giving rise to ESI-MS ions (not shown) consistent with a mixture of C-1 deuterated and non-deuterated components.

4. Conclusions

The glycoprotein extracted from conidia of *S. prolificans* (RMP-Sa-Coni) contained the same monosaccharide units as those of its mycelium (RMP-Sp), but with a trace of 2-*O*-methylrhamnose residues. Present were 2MeRha, Ara, Rha, Man, Gal, Glc, and GlcNH$_2$ in a molar ratio of 2:18:3:47:9:15:6, but there was a significant structural difference as mycelial RMP-Sp formed a precipitate with Cetavlon-borate, whereas RMP-Sp-Coni did not.

The oligosaccharide mixture, formed on reductive β-elimination of RMP-Sp-Coni, contained hexa-, penta-, and two tetrasaccharides, each containing 2-*O*-methylrhamnose as a capping group. After column chromatography, the most significant isolate was Fraction 1 consisting of epitope **1**, also obtained from mycelia of *S. prolificans* (Barreto-Bergter et al., 2008), epitope **2** with 2-*O*-methylrhamnose replacing the rhamnose capping group, and a pentasaccharide lacking the β-galactopyranosyl side-chain.

These results are significant, since 2-*O*-methyrhamnose has not yet been detected in fungi, although it has been widely encountered elsewhere. It occurs in protein-containing EPSs of the cyanobacteria (algae) *Microcoleus vaginatus*, *Scytonema javanicum*, *Phormedium tenue*, and a *Nostoc* sp. (Hu, Liu, Paulsen, Petersen, & Klavness, 2003), as well as a polysaccharide component in *Chlorella vulgaris* (Ogawa, Yamaura, & Maruyama, 1997). It has also been found in cells of *Bacillus anthracis* and *Bacillus cereus* (Fox, Black, Fox, & Rostovtseva, 1993). Terminal 2-*O*-methylrhamnose was shown to be linked to mannose residues in a glycoprotein from the bacterium *Flavobacterium columnare* (Vinogradov, Perry, & Kay, 2003) and to occur as 2-OMe-α-L-Rha*p*-(1 → 2)-α-L-Rha*p* groups in the surface glycopeptides of *Geobacillus stearothermophilus* (Schaffer et al., 2002). Units of 2-*O*-methylrhamnose are present in the antibiotics scopamycin A (McAlpine, Corcoran, & Egan, 1971) and lipiarmycin (Martinelli, Faniuolo, Tuan, Gallo, & Cavalerri, 1983).

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