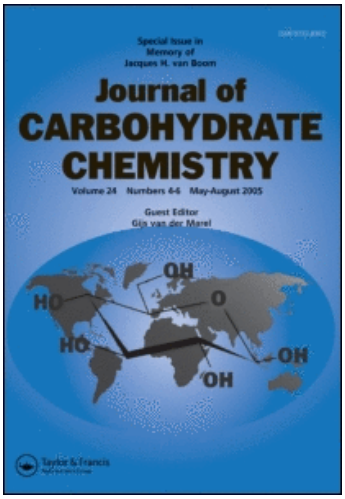


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Structure of Glucomannan-Protein from the Yeast *Cryptococcus Laurentii*

Nadežda Kolarova[a]; Mária Matulová[a]; Peter Capek[a]
[a] Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic

To cite this Article Kolarova, Nadežda , Matulová, Mária and Capek, Peter(1997) 'Structure of Glucomannan-Protein from the Yeast *Cryptococcus Laurentii*', Journal of Carbohydrate Chemistry, 16: 4, 609 — 623
To link to this Article: DOI: 10.1080/07328309708007339
URL: http://dx.doi.org/10.1080/07328309708007339

J. CARBOHYDRATE CHEMISTRY, 16(4&5), 609-623 (1997)

STRUCTURE OF GLUCOMANNAN-PROTEIN FROM THE YEAST *CRYPTOCOCCUS LAURENTII*

Nadežda Kolarova,* Mária Matulová, and Peter Capek

Institute of Chemistry, Slovak Academy of Sciences
842 38 Bratislava, Slovak Republic

Final Form February 19, 1997

ABSTRACT

The structure of an extracellular glucomannan-protein produced by *Cryptococcus laurentii* was studied. The glucomannan-protein was isolated via its insoluble copper complex. It was homogeneous on free-boundary electrophoresis, contained 91% saccharide, 6.5% protein and 1% phosphorus. It had $\bar{M}_n$ 21,000. The carbohydrate portion was composed of D-mannose and D-glucose in 33:2 molar ratio. From the results of compositional and methylation analyses, conventional acetolysis, as well as ^1H and ^{13}C NMR spectroscopy it was concluded that the glucomannan has an α-(1→6)-linked D-mannopyranosyl backbone having most residues (about 83%) substituted at O-2 with one, two, three or four D-mannopyranosyl units connected by α-(1→2) and α-(1→3) linkages. Moreover, an additional side chain with the α-D-Man*p*-(1→3)-D-Man*p*-(1→2)-D-Man*p*-(1→2)-D-Man*p* backbone structure in which α-D-glucopyranose residue is linked to O-2 of the mannopyranose unit next to the reducing end. Alkali treatment of glucomannan-protein in the presence of sodium borohydride showed that 87% serine and 83% threonine residues were glycosylated with mannose, mannobiose, and mannotriose.

INTRODUCTION

Cryptococcus laurentii is a zoopathogenic yeast-like organism and is closely related to the human pathogen *Cryptococcus neoformans* that causes opportunistic infection

affecting AIDS patients and transplant recipients. Extracellular carbohydrates play several roles in this infection.[2]

Fungi of the genus *Cryptococcus* are encapsulated nonfermenting yeast-like organisms. In addition to forming capsules, several species, among them *Cryptococcus laurentii,* excrete extracellular polysaccharides. Until now, it has been reported that *Cryptococcus laurentii* produced two different heteropolysaccharides that contained mannose residues. One of these is an extracellular polysaccharide composed of an α-(1→3)-linked mannan backbone with side chains containing terminal D-xylose and D-glucuronic acid residues.[3] The second one is a neutral polysaccharide composed of D-mannose, D-galactose, and D-xylose in which D-mannose residues are linked by α-(1→2) and α-(1→3) linkages.[4] Schutzbach and Ankel[5] provided evidence that *C. laurentii* contained at least four different mannosyl transferases. Three of these catalyze the formation of α-(1→2) and α-(1→3)-glycosidic linkages and α-D-mannopyranosyl-(1→2)-D-xylose linkage. The fourth mannosyl transferase catalyzes the formation of α-(1→6)-glycosidic linkage. Until now, α-D-mannopyranosyl-(1→6)-D-mannose linkage have not been described in the polysaccharides from *C. laurentii.*

The present work provides evidence that *C. laurentii* produces at least three different polysaccharides containing mannose residues. The isolation and characterization of the extracellular glucomannan-protein produced by *C. laurentii* are described.

RESULTS AND DISCUSSION

General properties. The glucomannan-protein (GMP) was isolated from the culture medium of *Cryptococcus laurentii* via its insoluble copper complex.[6] In moving boundary electrophoresis GMP exhibited a single sharp symmetrical peak. The average molecular mass of the GMP was determined to be 21,000. GMP contained 91% saccharide, 6.5% protein, 1.0% phosphorus, and consisted of D-mannose and D-glucose in 33:2 molar proportion. The absolute configuration of these sugar residues was determined by GLC of the trimethylsilyted (+)-2-butyl glycosides.[7] The optical rotation of GMP was +51° which suggests the prevalence of α-anomeric configuration of the saccharide units.

β-elimination reaction. To verify the presence of *O*-glycosidic linkages between the oligosaccharides and the protein, the GMP was treated with 0.1M NaOH in the presence of sodium borohydride. Under these conditions, *O*-glycosidically substituted serine and threonine residues are converted into alanine and α-aminobutyric acid. Quantitative analysis of the loss of serine and threonine, the increase in alanine, and the amount of α-aminobutyric acid showed that 87% serine and 83% threonine residues of GMP were

glycosylated (Table 1). Alkali treatment of MP afforded mainly mannobiose with low amount of mannose and mannotriose (Fig. 1).

Acetolysis of the glucomannnan. The conventional acetolysis of extracellular glucomannan yielded a mixture of mono- and oligosaccharides. The products were separated by gel filtration (Bio-Gel P-2) into six main fractions: **M-M5** and the fraction eluted in the void volume (Fig. 2). From the elution profile it is evident that major components of this acetolysate are fractions **M**, **M2**, **M3**, **M4**, and **M5**. Their mobilities on thin-layer chromatography (TLC, system S) were identical with those of the standards of mono-, di-, tri-, tetra-, and pentasaccharide of the α-(1→2) and α-(1→3)-linked α-D-mannose series. The amounts of higher oligomers **M6-M8** (not examined) were negligible. The acetolysate products eluted in void volume afforded on gel filtration (Bio-Gel P-4) two fractions M_X (M_n = 1574) and M_y (M_n not determined), containing 34.0% and 16.0% of protein, respectively. Compositional analysis of the individual fractions revealed the presence of D-mannose and D-glucose in **M**, **M4**, and **M5** and D-mannose in **M2**, **M3**, **Mx**, and **My**. While **M4** contained only a trace of D-glucose, **M** and **M5** contained D-mannose and D-glucose in molar ratios 3.5:1.0 and 5.2:1.0, respectively.

Methylation analysis of the glucomannan. Methylation analysis of GMP showed a variety of linkages and pointed to a highly branched structure of the polymer (Table 2). The detected derivatives imply that all saccharides are in the pyranose ring form. About 32% sugar units were involved in branching points. The derivatives originating from the polymer indicated the prevalence of 2- and 2,6-linked mannopyranose units. The content of 3-linked mannopyranose residues was much lower. Over 80% of 6-linked mannopyranose units were branched at O-2. Only minor portions of 2,3- and 3,6-linked mannopyranose units were found. The glucose derivatives pointed to the end position of this sugar and to units linked through O-6.

Methylation analysis of the glucomannan-derived oligomers. The results of the methylation analysis of the glucomannan-derived oligosaccharides are shown in Table 3. As can be seen from the table, the derivatives from the oligomers **M2**.-**M5** indicated the prevalence of terminal and 2-linked mannopyranose residues. Methylation analysis of the oligomers **M2-M5** showed that only **M5** was branched. The 3,4,6-tri-*O*-methyl- and 2,4,6-tri-*O*-methylmannose derivatives suggest that **M2** is a mixture of disaccharide isomers with (1→2) and (1→3) linkages in the ratio 9:1. The similar derivatives were found in oligosaccharides **M3** and **M4**, but the ratio of 2,4,6-tri-*O*-methyl- to 3,4,6-tri-*O*-methylmannose was increased. From these derivatives it was evident that **M3-M4** contain 2- and 3-linked D-Man residues. Methylation analysis of the oligomer **M5** afforded 2,3,4,6-tetra-*O*-methylglucose, 2,3,4,6-tetra-*O*-methyl-, 3,4,6-tri-*O*-methyl-, 2,4,6-tri-*O*-methyl-, and 4,6-di-*O*-methylmannose, confirming the nonreducing terminal positions of

Table 1. Changes in the concentration of amino acids present in GMP after β-elimination reaction.

Amino acid	before β-elimination μmol.g $^{-1}$	after β-elimination μmol.g $^{-1}$
Asp	29.64	26.05
Thr	68.47	8.81
Ser	76.08	13.05
Glu	30.72	28.68
Pro	18.41	16.92
Gly	18.47	20.69
α-aminobutyric acid	-	60.81
Ala	28.58	82.24
Val	24.11	25.72
Met	1.47	1.95
Ile	8.77	7.34
Leu	9.29	10.68
Tyr	8.57	7.13
Phe	6.22	7.25
His	3.94	6.98
Arg	traces	traces

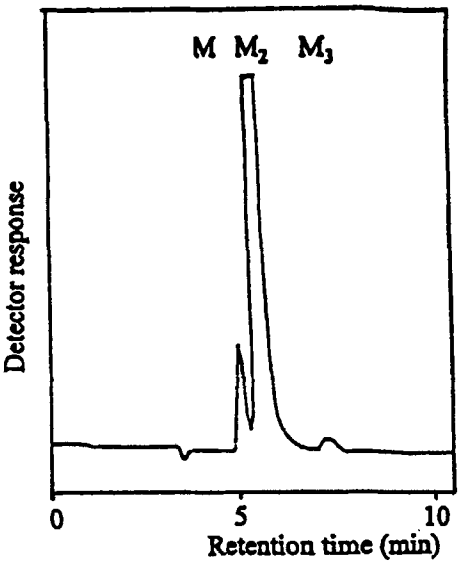


Figure 1. HPLC profile of the saccharides released by the alkali-induced β-elimination.

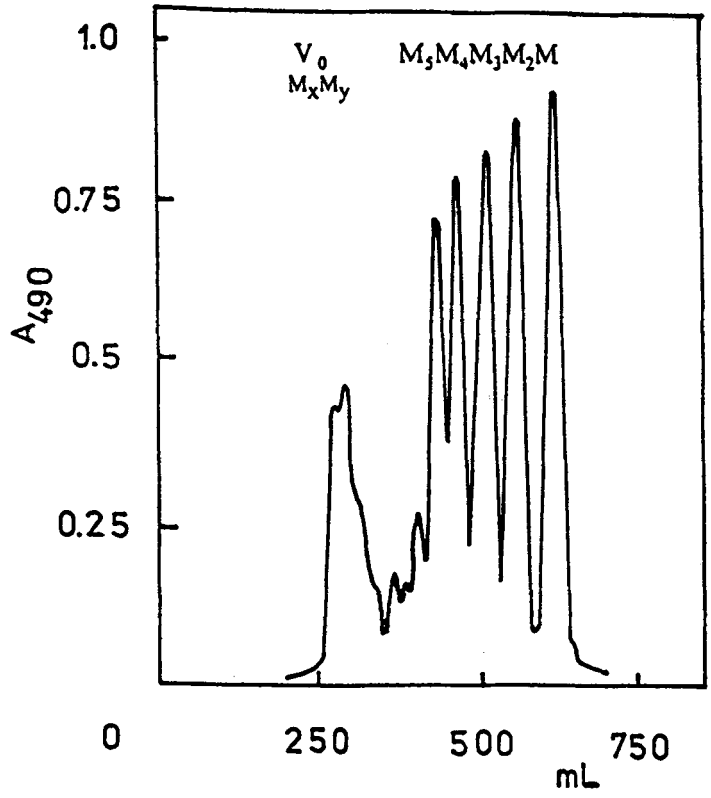


Figure 2. Gel filtration (Bio-Gel P-2) pattern of the oligosaccharides from acetolysis of extracellular GMP.

Table 2. Methylation analysis data of the glucomannan from *C. laurentii*.

Derivative	Mole%	Mode of linkage
2,3,4,6-Me_4-Man[a]	30.1	Man-(1→
2,3,4,6-Me_4-Glc	2.1	Glc-(1→
3,4,6-Me_3-Man	20.2	→2)-Man-(1→
2,4,6-Me_3-Man	6.9	→3)-Man-(1→
2,3,4-Me_3-Man	6.1	→6)-Man-(1→
2,3,4-Me_3-Glc	1.9	→6)-Glc-(1→
4,6-Me_2-Man	2.3	→2,3)-Man-(1→
3,4-Me_2-Man	30.4	→2,6)-Man-(1→
2,4-Me_2-Man	b	→3,6)-Man-(1→

a. 2,3,4,6-Me_4-Man = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol, etc., b. Traces.

Table 3. Methylation analysis data of the acetolysis products (M_2-M_5, M_x and M_y) of *C. laurentii* glucomannan.

Derivative	Compound (mole %)						Mode of linkage
	M_2	M_3	M_4	M_5	M_x	M_y	
2,3,4,6-Me_4-Man[a]	50.7	35.2	28.5	21.4	15.5	16.4	Man-(1→
2,3,4,6-Me_4-Glc	-	-	b	16.1	-	-	Glc-(1 →
3,4,6-Me_3-Man	44.4	48.0	51.5	39.9	48.6	49.1	→2)-Man-(1→
2,4,6-Me_3-Man	4.9	16.8	19.4	6.9	19.6	21.7	→3)-Man-(1→
2,3,4-Me_3-Man	-	-	-	b	3.9	-	→6)-Man-(1→
4,6-Me_2-Man	-	-	b	15.7	4.7	5.1	→2,3)-Man-(1→
3,4-Me_2-Man	-	-	-	-	7.7	6.5	→2,6)-Man-(1→
2,4-Me_2-Man	-	-	-	-	-	1.2	→3,6)-Man-(1→

a. 2,3,4,6-Me_4-Man = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol, etc., b. Traces

D-glucose and D-mannose residues, as well as presence of 2-, 3-, and 2,3-linked mannose units. The last mentioned derivative confirms the branched structure of M_5 (Table 4). The derivatives 2,3,4-tri-*O*-methyl- (3.9%) and 3,4-di-*O*-methylmannose (7.7%) originating from the higher oligomer M_x and 3,4-di-*O*-methyl- (6.5%) and 2,4-di-*O*-methylmannose (1.2%) from M_y implied that some (1→6)-glycosidic linkages have not been completely cleaved by acetolysis. The content of dimethylated derivatives, i.e. 4,6-di-*O*-methyl- (4.7%) and 3,4-di-*O*-methylmannose (7.7%) derived from M_x, and 4,6-di-*O*-methyl- (5.1%), 3,4-di-*O*-methyl- (6.5%), and 2,4-di-*O*-methylmannose (1.2%) derived from M_y pointed to branched structure of these compounds.

NMR spectroscopy of oligomers and the native polymer. ^1H and ^{13}C NMR spectra of the fractions M_2-M_5 are presented on Figs. 3 and 4. Assignment of the H1 and H2 signals in the ^1H NMR spectra was performed on the basis of the data presented by Cohen and Ballou.[8] In full agreement with the reference data of the α-D-Man-(1→2)-D-Man disaccharide, in the M_2 spectrum the H1 signals of α- and β-Man of the reducing end were found at δ 5.38 and 4.92, respectively, while the anomeric signal of the terminal nonreducing mannose residue was present at δ 5.04. Overlapping signals at δ 5.15 due to

Table 4. H1 and H2 chemical shifts of oligosaccharides released by acetolysis of *C. laurentii* glucomannan.

	Sugar residues					Chemical shift of residue				
	E	D	C	B	A		E	D	C	B	A
M_2				α-D-Man-(1→2)-D-Man		H1				5.04	5.38
						H2				x	4.07
				α-D-Man-(1→3)-D-Man		H1				5.15	5.15
						H2				x	4.11
M_3			α-D-Man-(1→2)-D-Man-(1→2)-D-Man			H1			5.04	5.32	5.38
						H2			4.07	4.10	x
			α-D-Man-(1→3)-D-Man-(1→2)-D-Man			H1			5.16	5.03	5.38
						H2			4.07	4.22	x
M_4		α-D-Man-(1→3)-D-Man-(1→2)-D-Man-(1→2)-D-Man				H1		5.16	5.04	5.30	5.38
						H2		4.07	4.22	4.10	3.92
M_5		α-D-Man-(1→3)-D-Man-(1→2)-D-Man-(1→2)-D-Man				H1		5.13	5.30	5.28	5.38
		2 ↑ 1				H2		4.07	4.22	4.09	3.92
			α-D-Glc			H1			5.21		
						H2			3.52		
	α-D-Man-(1→3)-D-Man-(1→2)-D-Man-(1→2)-D-Man-(1→2)-D-Man					H1	5.13	5.11	5.04	5.30	5.38
						H2	4.10	4.20	4.10	4.10	x

x - not assigned

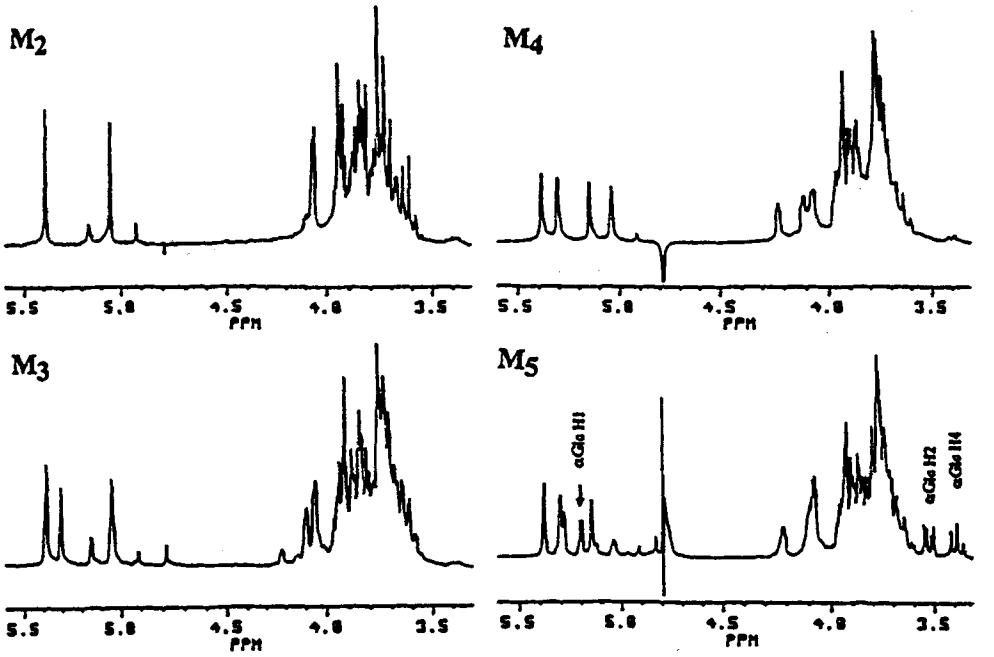


Figure 3. ^{1}H NMR spectra of M_2-M_5.

the terminal Man and αMan of the reducing end suggested the presence of a low amount of α-D-Man-(1→3)-D-Man disaccharide (~10%).

For the predominant compound in the **M3** spectrum, chemical shifts of the H1 Man signals were present at δ 5.38, 5.32 and 5.04 and those of H2 were found at δ 4.08 and 4.12. These data were in accordance with those of α-D-Man-(1→2)-α-D-Man-(1→2)-D-Man trisaccharide.[8] The presence of low amount of further trisaccharide was revealed by the H1 signal at δ 5.16 due to the terminal Man linked to O-3 of the neighbouring Man residue, the H2 signal of which was found at characteristic value of δ 4.22. On the basis of the integral intensities and chemical shift values of the H1 signals, the fraction **M3** was identified as a mixture of two trioses, i.e, α-D-Man-(1→2)-α-D-Man-(1→2)-D-Man and α -D-Man-(1→3)-α-D-Man-(1→2)-D-Man in the ratio 3:1. A simple comparison of the H1 and H2 chemical shifts in the fraction **M4** spectrum with the data of already published different mannooligosaccharides[8,9] gave evidence that in **M4** α-D-Man-(1→3)-α-D-Man-(1→2)-α-D-Man-(1→2)-D-Man is present. In the ^{13}C NMR spectra of **M2** and **M3** (Fig. 4), the chemical shifts of the signals were in egreement with the reference data.[10] In the spectrum of **M4**, the C1 signal of the nonreducing terminal unit was found at δ 102.9,

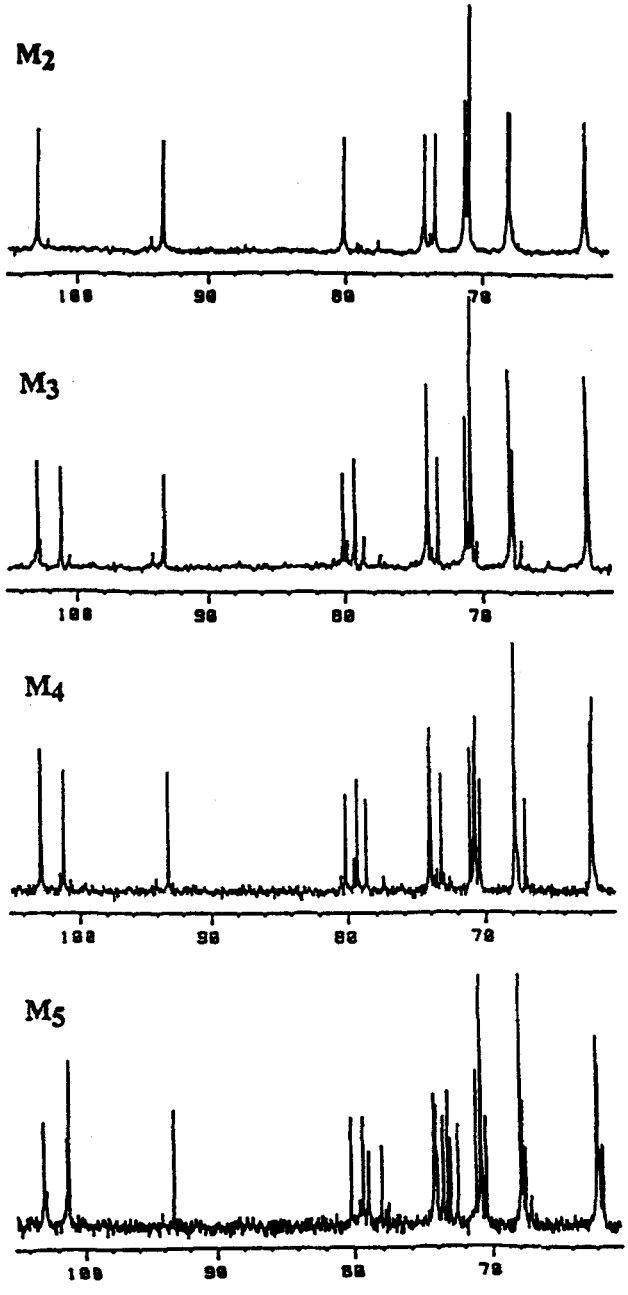
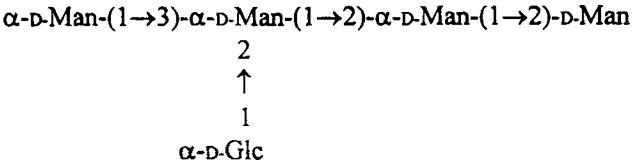


Figure 4. ^{13}C NMR spectra of M_2-M_5.

while that one of its neighbouring 3-linked α-D-Man appeared at δ 102.8. The C1 chemical shifts of the Man unit next to the reducing end and the signal of the reducing end α-D-Man were 101.2 and 93.2, respectively.

Intensities of the anomeric signals in the ^{1}H NMR spectrum of **M_5** suggested again a mixture of two pentasaccharides. In comparison with the **M_2-M_4** spectra a new signal at δ 5.21 appeared in the **M_5** spectrum. This signal was attributed to α-Glc residue because of its $^3J_{1,2}$=3.9 Hz value as well as an easy polarization transfer in the 2D relayed COSY spectra (δ (J/Hz): H2 3.52 (9.6), H3 3.74 (9.8), H4 3.37 (9.6) which is characteristic of the gluco configuration. In comparison with α-Glc H1 signal, the H1 signal intensity at δ 5.16 of the terminal mannose linked to O-3 of the neighbouring mannose indicated that this type of linkage is present in both pentasaccharides. The content of the minor component in the **M_5** mixture was indicated by intensity of the signal at δ 5.04, due to the 3-linked mannose next to the terminal mannose. The other signals were overlapped with those belonging to the predominant pentasaccharide at δ 5.38, 5.31 and 5.16. Chemical shifts of the signals of this minor component point to the α-D-Man-(1→3)-α-D-Man-(1→2)-α-D-Man-(1→2)-α-D-Man-(1→2)-α-D-Man-(1→2)-D-Man pentasaccharide structure. For the predominant compound in the **M_5** spectrum the chemical shift of the H1 Man signals at δ 5.28 and 5.30 were characteristic of internal 2-linked α-D-Man residue.[8] However, they differed in the H2 chemical shifts (Table 4). The H2 chemical shift at δ 4.22 is characteristic of the 3-linked mannose unit next to the terminal Man so the H1 signals at δ 5.30 have been attributed to the 3-linked α-D-Man branched at O-2 by α-D-Glc unit. These facts as well as results of methylation analysis suggest the following structure of the predominant pentasaccharide presented in **M_5**



Signals in the ^{1}H NMR spectra of the higher molecular mass fractions **M_X** and **M_Y** had broad shape, probably due to the presence of the protein. Results of methylation analysis (Table 3) showed a large variety of linkage types in both fractions which was reflected also in the distribution of the signals in the anomeric region of the spectra. In the **M_Y** ^{1}H NMR spectrum (Fig. 5a), overlapping signals at δ 5.16 and 5.13 suggest the presence of terminal nonreducing mannose units linked to O-3 of the neighbouring mannose residues, while those around δ 5.37 indicate internal 3-linked α-D-Man units[9] in addition to the mannose unit at the reducing end. The signals around δ 5.30 are characteristic of the internal 2-linked α-D-Man residues. The H1 signal at δ 5.04 indicate

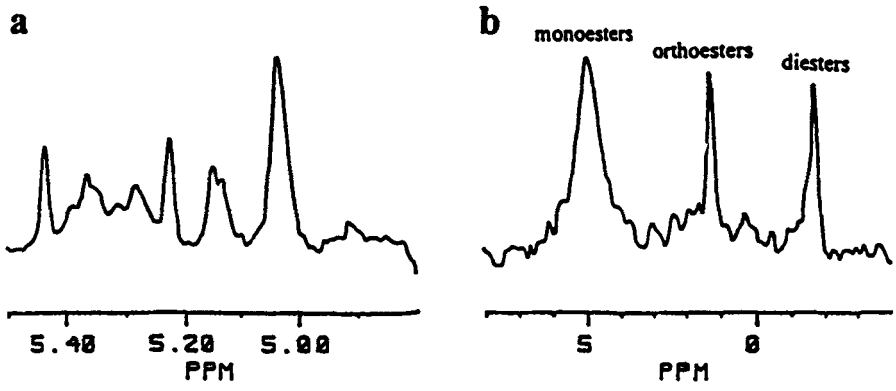


Figure 5. Spectra of M_y: a - anomeric region of the ^1H NMR spectrum, b - decoupled ^{31}P NMR spectrum.

the presence of terminal nonreducing α-D-Man linked to O-2 of the neighbouring Man as well as that of the 2-linked α-D-Man next to the 3-linked α-D-Man. No signals bellow δ 5.00 were observed in the spectra and thus the presence of the β-linked Man[11] in the fraction M_y could be excluded.

In comparison with the M_2-M_5 spectra in the M_y spectrum, a new signal at δ 5.43 appeared. In the tri- and tetrasaccharides derived from the yeast mannan,[12] with the structure equivalent to M_3 and M_4, an O-6-linked phosphate group was found at the 2-linked α-D-Man next to the reducing end. The H1 chemical shift changes for phosphorylated mannose unit from δ 5.300 to 5.447 and for reducing end Man from δ 5.371 to 5.235 were observed in the ^1H NMR spectra of the oligosaccharides.[12] On the basis of this fact, the H1 signal at δ 5.43 in the M_y spectrum suggests the presence of the O-6-linked phosphate group.

In fact, in the ^{31}P NMR spectrum of M_y (Fig. 5b) three different phosphorus signals were detected. The value of the chemical shift of the signal at δ 4.90 is in very good accordance with the values of the typical ^{31}P NMR chemical shifts[13] for O-6-linked phosphate which were found in the range of δ 4.54 - 3.90. The other two signals at δ 1.15 and -1.82 could be identified as inorganic orthophosphate and diesterified phosphate, respectively. In the case of the M_x fraction neither the ^1H NMR spectrum nor the ^{31}P NMR spectrum implied a significant content of phosphorus.

In the anomeric region of the ^1H NMR spectrum of the native polymer (Fig. 6a) three major types of signals could be identified. The signal at δ 5.04 suggested that the large part of the terminal mannose units was linked to O-2 of the neighbouring mannose,

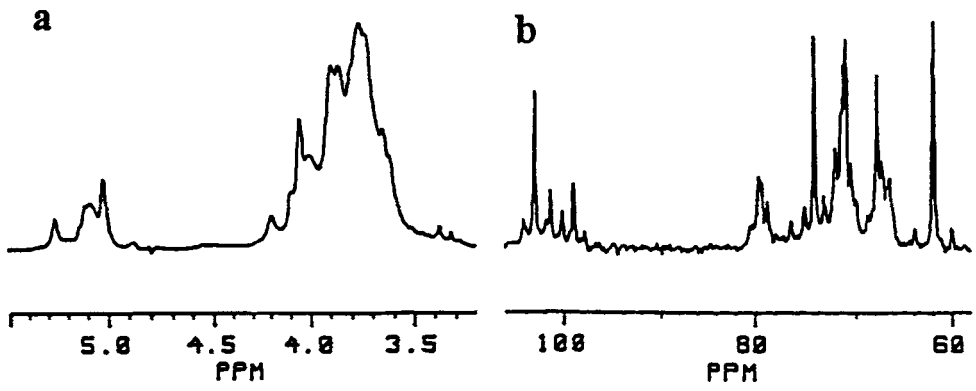


Figure 6. a - ^{1}H NMR spectrum of GMP, b - ^{13}C NMR spectrum of GMP.

while a small shoulder at δ 5.14 indicated that a minor part of the terminal mannose unit is linked to O-3. Overlapping signals at δ 5.11 and 5.09 could be due to 6-linked mannose residues while the signal at δ 5.27 indicated internal 2-linked mannose units. All these signals reflected a highly branched structure of the polymer. Moreover, a signal of small intensity at δ 4.83 suggested the presence of the small number of the β-linked mannose residues which might explain a relatively lower value (+51°) of the optical rotation of the polymer.

The results of chemical and spectroscopic analyses indicate a highly branched comb-like structure of the extracellular glucomannan. The main chain of GMP consists of 6-linked α-D-mannopyranose residues, about 83% of which are substituted exclusively at O-2 by the side oligosaccharide chains. Acetolysis products of GMP showed that the side chains contained linear and branched oligosaccharides comprised of 2- and 3-linked mannopyranose residues. The branched tetrasaccharide was the only isomer that carried at O-2 of 3-linked D-mannopyranose residue (next to the terminal unit), a nonreducing α-D-glucopyranose unit. A small amount of higher oligomers produced by acetolysis of GMP indicated that extracellular glucomannan of *Cryptococcus laurentii* contains predominantly short side oligomeric chains (di- tri-, and tetrasaccharide isomers). The phosphate groups were located in the side chains at O-6 of some mannopyranose units linked to the main chain.

The resultes presented in this paper demonstrate that *C. laurentii* produces glycoprotein, the backbone of which consists of α-(1→6)-linked mannopyranose residues. The isolated glucomannan resembles cellular glucomannan of *Candida utilis*.[14]

EXPERIMENTAL

Production, isolation, and purification of the *Cryptococcus laurentii* GMP. *Cryptococcus laurentii* CCY 17-3-5 from the Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, was grown at 28 °C in semisynthetic liquid medium that contained per 1 L: 2g glucose, 0.3g $(NH_4)_2SO_4$, 0.1g $MgSO_4$, 0.05g KH_2PO_4, 0.3g yeast autolysate. After 1 week, harvested cells were separated from the medium by centrifugation (8,000g, 20 min, 4 °C). Extracellular glycoproteins were obtained by precipitation of the supernatant with ethanol. The resulting precipitate was dissolved in distilled water and GMP was isolated from solution via its insoluble copper complex.[5]

Molecular mass determination. The number average molecular mass (M_n) was determined osmometrically at 30 °C, using a Knauer Vapour Pressure Osmometer.

Free boundary electrophoresis. Free-boundary electrophoresis of a 1% solution of the extracellular GMP was carried out in a Zeiss 35 apparatus, using 0.05M sodium tetraborate buffer (pH 9.2) at 150 V/cm and 6 mA, for 30 min.

Protein assay. Protein content was estimated by the method of Lowry[15] using bovine serum albumin as a standard.

Monosaccharide analysis. The constituent monosaccharides of the extracellular glycoprotein were identified after hydrolysis (2 M trifluoroacetic acid, 2 h, 100 °C) and reduction, in the form of their trifluoroacetates[16] by gas chromatography on a Hewlett-Packard Model 5890 Series II instrument equipped with a PAS 1701 column (0.32 mm x 25 m) at the temperature program of 110-125 °C (2 °C/min)-165 °C (20 °C/min) and flow rate of nitrogen 20 mL/min.

β-elimination reaction. Glycoproteins were treated with 0.1 M NaOH in the presence of 1 M $NaBH_4$ at 40 °C for 16 h.

Amino acid analysis. The amino acid composition was established with an automatic amino analyser, type 6020 (Mikrotechna, Prague), after hydrolysis of the glycoprotein (6 M HCl, 20 h, 100 °C).

HPLC and TLC analysis of the oligosaccharides.. HPLC analysis of β-elimination reaction products was performed on Hewlett-Packard 1050 liquid chromatograph equipped with a refractometer and an SGX C18 TESSEK column (0.4 cm x 25 cm). Thin-layer chromatography (TLC) was carried out on Kieselgel 60 in the solvent system S, 1-butanol - formic acid - water 2:3:1. The saccharides were visualized by spraying the plates with 20% ammonium sulfate and heating.

Acetolysis of the glucomannan. The procedure of Kocourek and Ballou[17] was used. The mixture of deacetylated products was fractionated on a column (2.5 x 200 cm)

of Bio-Gel P-2 or P-4. The compounds were eluted with water at flow rate of 10 mL/ h. Fractions of 2 mL were collected and analyzed for the carbohydrate content by phenol-sulfuric acid assay.[18]

Sugar linkage analysis. The dry samples of oligo- and polysaccharides (3-4 mg) were solubilized in dry dimethyl sulfoxide (1 mL) and methylated by the Hakomori method.[19] The methylated products were isolated by partition with dichloromethane, concentrated and hydrolyzed with 2 M trifluoroacetic acid (1 h, 120 °C). The partially methylated monosaccharides were reduced with sodium borodeuteride, acetylated and analyzed by gas chromatography-mass spectrometry. GLC-MS of partially methylated alditol acetates was effected on a FINNIGAN MAT SSQ 710 spectrometer equipped with an SP 2330 column (0.25 mm x 30 m) at 80-240 °C (6 °C/min), 70 eV, 200 μA, and ion-source temperature 150 °C.

NMR spectroscopy. ^{1}H and ^{13}C NMR spectra were measured in deuterium oxide at 298 K on a Bruker AM-300 spectrometer. Chemical shifts in the ^{1}H NMR spectra were referenced to the HOD signal at 4.78 ppm with respect to external acetone (2.225 ppm) and in the ^{13}C NMR spectra with respect to external acetone at 31.07 ppm. The samples of oligosaccharides (ca. 5-8 mg) were lyophilized 2-3 times from D_2O.

ACKNOWLEDGEMENTS

We thank to Mr. V. Hladký and Ms. E. Marková for technical assistances and to Dr. G. Kogan for proof-reading the manuscript. This work was supported in part by the Grants No.2/1240/95 and 2/1233/96 from the Slovak Scientific Grant Agency.

REFERENCES

1. Presented at the *XVIIIth International Carbohydrate Symposium,* Milan, Italy, July 21-26, 1996.
2. C. W. White and E. S. Jacobson, *Can. J. Microbiol.*, **39**, 129 (1992).
3. R. J. Stoodley, *M. A. Thesis*, Queens University, Kingston, Ontario, Canada, 1959.
4. H. Ankel, E. Ankel, J. S. Schutzbach, and J. C. Garancis, *Can. J. Biochem.*, **36**, 1617 (1970).
5. J. S. Schutzbach and H. Ankel, *J. Biol. Chem.*, **246**, 2187 (1971).
6. S. Šikl, M. Masler and S. Bauer, *Collect. Czech.. Chem.Commun.*, **35**, 2965 (1970).
7. G. J. Gerwik, J. P. Kamerling and J. F. G. Vliegenthart, *Carbohydr. Res.*, **77**, 377 (1979).
8. R. E. Cohen and C. E. Ballou, *Biochemistry,* **19**, 4345 (1980).

9. N. Shibata, Ch. Kojima, Y. Satoh, R. Satoh, A. Suzuki, H. Kobayashi, and S. Suzuki, *Eur. J. Biochem.*, 217, 1 (1993).
10. T. Ogawa and H. Yamamoto, *Carbohydr. Res.*, **104**, 271 (1982).
11. W. Zhang and C. E. Ballou, *J. Biol. Chem.*, **256**, 10073 (1981).
12. P. de Waard, J. F. G. Vliegenthart, Y. Kozutsumi, T. Kawasaki, and I. Yamashina, *J. Biol. Chem.*, **264**, 12141 (1989).
13. A. J. R. Costello, T. Glonek, M. E. Slodki, and T. R. Seymonn, *Carbohydr. Res.*, **42**, 23, (1975).
14. G. Kogan, J. Šandula, and V. Šimkovicova, *Folia Microbiol.*, **38**, 219 (1994).
15. O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
16. J. Shapira, *Nature*, **222**, 792 (1969).
17. J. Kocourek and C. E. Ballou, *J. Bacteriol.*, **100**, 1175 (1969).
18. M. Dubois, K. A. Gilles, K. J. Hamilton, P. A. Rebers, and E. Smith, *Anal. Biochem.*, **28**, 350 (1959).
19. S. Hakomori, *J. Biochem. (Tokyo)*, **55**, 205 (1964).