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CELL CONSTITUENTS OF MYCELIA AND CONIDIA OF *Aspergillus fumigatus*

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ABSTRACT

Cell-wall components of mycelia and conidia of *Aspergillus fumigatus* contain alkali-soluble polysaccharides comprised of D-galacto-D-mannans which coprecipitated with small proportions of a D-glucan, tentatively identified as glycogen. The fine structures of the D-galacto-D-mannans varied as a function of the cell type. In a 5-day-old mycelium, the polysaccharide consisted of a main chain of (1→6)-linked α-D-mannopyranose residues substituted at O-2 by 1 to 3 α-D-mannopyranosyl units that are (1→2)-interlinked. β-D-Galactofuranosyl units are (1→6)-linked to the D-mannan core, being components of side-chains of average length of ~6 units, which are (1→5)-interlinked. The 10-day-old mycelium had a similar D-galacto-D-mannan, but the proportion of glycogen was smaller. Conidia contain polysaccharides of different structure, as shown by the ^{13}C-n.m.r. spectrum and by methylation analysis. Side chains composed of a single unit of β-D-galactofuranosyl linked (1→6) to adjacent D-mannopyranosyl units were identified with a minor proportion of 6-*O*-substituted D-galactofuranosyl units. Also present were nonreducing D-galactopyranosyl end-groups and 2-amino-2-deoxyglycosyl units. The glucan component was not glycogen. Conidial walls have much less protein than mycelial walls. Predominant amino acids in the latter were aspartic and glutamic acids, tyrosine, alanine, and glycine. Fatty acids C_{16}, C_{18}, $C_{18:1}$, and $C_{18:2}$ were present in the mycelial and conidial walls, $C_{18:2}$ was present in minor amounts in the mycelial wall, but was a major component of the lipid fraction from whole cells.

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INTRODUCTION

Aspergillus fumigatus is a pathogenic fungal species that causes allergic aspergillosis and aspergilloma in humans. Antigenic preparations consisting of culture filtrates, and mycelial and cell-wall extracts were shown to react with sera from patients with aspergillosis[1-4]. An *Aspergillus*-active protein was obtained which elicited delayed-type skin-reactions[5]. Azuma *et al.*[6] purified a glycopeptide antigen from *A. fumigatus* consisting of 94% sugar and 6% peptide. The carbohydrate part of this antigen was a D-galacto-D-mannan that cross-reacted serologically with D-galacto-D-mannans from *A. flavus*, *A. niger*, *A. effusus*, and several *Penicillium* species[6]. The immunologically-active D-galacto-D-mannans react with antisera that are specific for D-galactofuranosyl residues[6-8]. Partial structures of the D-galacto-D-mannans from *A. niger* and *A. terreus*, and *A. flavus*, *A. nidulans*, and *A. niger* have interrelated chemical structures. The D-galacto-D-mannan from *A. niger* consists of a main-chain of (1→6)-linked α-D-mannopyranosyl residues and side-chains composed of (1→5)-linked β-D-galactofuranosyl and (1→2)-linked α-D-mannopyranosyl residues[9]. In the present study, we determined and compared the fine structures of the D-galacto-D-mannans obtained from the mycelium and conidia of *A. fumigatus*. We also studied the distribution of other cell-wall constituents in order to discern differences that could be associated with different morphological cell-types of *A. fumigatus*.

EXPERIMENTAL

Microorganisms. — *Aspergillus fumigatus* (NCPF 2140) was provided by Dr. D. W. R. Mackenzie from the Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine. A preparation of isolated conidia was kindly provided separately by the same laboratory. The *Aspergillus* strain was maintained in a medium containing (g/L): peptone (Difco) 10, yeast extract (Difco) 5, agar (Difco) 20, and D-glucose 40. The incubation was performed for 72 h at 25°, and the culture was kept at 4° under a layer of mineral oil. The transfers were made each 6 months. Hyphae were obtained on a medium (*A*) at pH 6.5 containing (g/L): D-glucose 20, peptone 10, and a dialyzate of yeast extract 5.

Isolation of hyphae. — The cells, grown on solid Sabouraud medium, were inoculated into 500 mL-Erlenmeyer flasks containing medium *A* (200 mL). The flasks were incubated for 3–4 days at 25°, with shaking. These cultures were then transferred to 3 L of the same medium distributed equally between Fernbach flasks. The final cultures were incubated at 25° for 5 or 10 days, and the mycelia collected by centrifugation, dispersed in water, and recentrifuged, the washing being repeated 3 times. The washed cells were lyophilized. The yield of dry mycelium was 8.6 g/L of 10-day culture and 6.9 g/L of 5-day culture.

Extraction and purification of polysaccharides. — Polysaccharides were extracted from intact hyphae of *A. fumigatus* (1 g) with hot 6% aqueous potassium hydroxide (200 mL) for 6 h at 100°. The extract was neutralized with acetic acid and centrifuged,

and the supernatant was concentrated to a small volume. The polysaccharide was precipitated with excess ethanol, and the precipitate washed with ethanol and dried. A solution of polysaccharide in water was passed through a column of Amberlite IR-120 (H^+) and Dowex 1-X8 (OAc^-) ion-exchange resins. The eluate was concentrated to a small volume, and the polysaccharide was precipitated with ethanol. Yields were 8 mg of polysaccharide/g of dry cells from a 10-day culture, and 7.2 mg/g cells from a 5-day culture. One gram of the dry conidia, obtained separately, yielded 21 mg of purified polysaccharide.

Component sugars of polysaccharides. — Polysaccharides were hydrolyzed with 0.5M sulfuric acid for 18 h at 100°. The resulting monosaccharides were characterized as their derived alditol acetates by g.l.c. according to Sawardeker *et al.*[10].

Methylation of polysaccharides. — Polysaccharides were methylated by the method of Haworth[11]. The partially methylated products were then isolated by dialysis and evaporation, and then completely methylated by the procedure of Kuhn *et al.*[12]. A solution of the per-*O*-methylated polysaccharide in methanol containing 3% hydrogen chloride was boiled under reflux for 18 h, the acid neutralized with silver carbonate, and the supernatant filtered and evaporated to a syrup. Complete hydrolysis was effected with 10% aqueous sulfuric acid for 18 h at 100°. The product was reduced with sodium borohydride and acetylated. Partially *O*-methylated alditol acetates were identified by use of a g.l.c. unit coupled to a mass spectrometer (electron impact[13]) equipped with a 30-m glass capillary column filled with either OV-225 or OV-17, and programmed from 95 to 230° (at 2°/min). The more polar OV-225 provided a better resolution of the peaks, but better quantitative results were obtained with OV-17, as the less volatile components were more readily eluted.

Partial acetolysis. — This procedure was performed as described by Lee and Ballou[14]. The sugars obtained on *O*-deacetylation were examined by paper chromatography with 2:1:1 (v/v) 1-butanol–ethanol–water, the spots being detected with *p*-anisidine hydrochloride[15] and ammoniacal silver nitrate[16] sprays.

Degradation of polysaccharides. — A sample of the polysaccharide (5 mg) was dissolved in water (5 mL), acidified to pH 2 or 1.8, and heated for 4 h at 100°. The degraded polysaccharide (3 mg) was dialyzed, lyophilized, and treated with α-D-mannosidase from *Arthrobacter* sp GJM-1[17], as previously described[9].

^{13}C-N.m.r. spectroscopy. — Spectra were obtained as previously outlined[18], from polysaccharide samples dissolved in deuterium oxide (2 mL) at 70° in a 12-mm diameter tube. Chemical shifts are expressed as δ relative to an external standard of tetramethylsilane, the resonance of which was determined in a separate experiment.

Cell-wall preparations. — Cell walls were prepared from dry cells (1 g) as described elsewhere[19]; yield: 160 mg of cell-wall material from 1 g of 10-day-old mycelium; 70 mg from 1 g of 5-day-old mycelium; and 88 mg from 1 g of dry conidia.

Cell-wall components. — The protein content was determined by quantitative amino acid analysis of purified cell-wall preparations and of whole cells. Samples (5–7 mg) were hydrolyzed with 5.7–6M hydrochloric acid for 18 h at 105°. Analyses were performed with a Beckman amino acid analyzer. Lipids were determined by

the method of Bartnicki-Garcia and Nickerson[20], and separated into Fractions I and II as described by Previato *et al.*[19]. Fatty acids were determined according to the method of Tulloch[21]: the methyl esters were prepared by boiling under reflux for 3 h in 2.5% methanolic hydrogen chloride; after cooling and mixing the solution with water, the methyl esters were extracted with chloroform. The extracts were analyzed by g.l.c. with conventional columns containing 15% of OV-275 on Supelco DMCS-PQW Chromosorb (100–120 mesh) at 190°, and 3% of Dexsil 300 on Chromosorb W (80–100 mesh)-DMCS AW, 125–300° (3°/min). The methyl esters were identified by their retention times and by the *m/z* values obtained by g.l.c.–m.s.

RESULTS

The content of the neutral carbohydrate components of the 5- and 10-day-old mycelia, as well as of the isolated conidia of *A. fumigatus*, for whole cells, cell-wall preparations, and purified polysaccharides are reported in Table I.

Polysaccharide from the mycelium of a 5-day culture. — The polysaccharide (see Table I) obtained at 25° had $[\alpha]_D^{25}$ +18° (*c* 0.3, water). Methylation followed by hydrolysis, sodium borohydride reduction, and acetylation gave *O*-methylalditol acetates as shown in Table II. A superior separation occurred by use of OV-225, but the less-volatile components were not completely eluted, and the percentage composition obtained with OV-17 was higher. The derivatives were characterized by their retention times and mass spectrometric patterns on electron impact[13]. The highly branched structure contained D-galactofuranosyl and D-mannopyranosyl nonreducing end-groups, 2-*O*- and 6-*O*-substituted D-mannopyranosyl, 4-*O*-substituted D-galactopyranosyl or 5-*O*-substituted D-galactofuranosyl, and 2,6-di-*O*-substituted D-mannopyranosyl residues. D-Glucopyranosyl units were present as nonreducing end-groups, and 4-*O*- and 4,6-di-*O*-substituted residues, different from those obtained from nigeran produced by *A. niger*[9].

D-Galactosyl units were partly removed by hydrolysis of the polysaccharide

TABLE I

NEUTRAL CARBOHYDRATE COMPONENTS OF WHOLE CELLS AND CELL FRACTIONS OF MYCELIA AND CONIDIA OF *A. fumigatus*[a]

Monosaccharide	*5-day-old Mycelium*			*10-day-old-Mycelium*			*Conidia*		
(%)	*WC*	*CW*	*P*	*WC*	*CW*	*P*	*CW*	*WC*	*P*
D-Mannose	42	13	25	28	20	40	18.5	28	64
D-Galactose	12	18	41	9	18	40	9	14	25
D-Glucose	45	69	34	60	61	20	72	57	11

[a]Abbreviations: WC, whole cells; CW, cell walls; and P, polysaccharides extracted as described in the Experimental section.

TABLE II

PERCENTAGES OF *O*-METHYLALDITOL ACETATES OBTAINED FROM POLYSACCHARIDE FRACTIONS OF MYCELIA AND CONIDIA OF *A. fumigatus*[a]

O-Methylalditol acetate	*Source of polysaccharide*									
	5-Day-old mycelium		*5-Day-old mycelium*		*10-Day-old mycelium*		*10-Day-old mycelium*		*Conidia*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
2,3,4,6-Me_4-Man	11	6	18	12	14	9	19	12	14	7
2,3,4,6-Me_4-Glc		4		8		3		3		3
2,3,5,6-Me_4-Gal	7	6	5	5	5	8	6	4	18	13
2,3,4,6-Me_4-Gal									2	3
3,4,6-Me_3-Man	13	15	34	26	5	16	17	38	20	18
2,4,6-Me_3-Man	2	1	3	2	3	2	5	3		
2,3,6-Me_3-Gal	32	34			38	38		1		
2,3,6-Me_3-Glc		24		25		10		11		2
2,3,4-Me_3-Man	18		24	8	21		31	15	24	18
2,3,4-Me_3-Glc										
2,3,5-Me_3-Gal									1	2
3,4-Me_2-Man	14	5	13	9	10	12	12	11	17	9
2,3-Me_2-Glc	3	4	3	5[b]	4	2	10[b]	2	4	8[b]

[a]Peak areas (% of total) of *O*-methylalditol acetates on columns of (A) OV-17 and (B) OV-225. [b]High values probably due to overlapping with peak of noncarbohydrate component.

from a 5-day-old culture, for 4 h at 100° at pH 2, providing a degraded polymer that was isolated after the low-molecular-weight degradation products were removed by dialysis. Hydrolysis gave D-mannose, D-galactose, and D-glucose in the ratio of 47:25:28 (compare with 25:41:34 of starting material). Methylation analysis (Table II) showed results similar to those obtained for galactomannan of *A. niger* degraded by partial hydrolysis, all D-galactofuranosyl residues, as represented by the 2,3,5,6-tetra-*O*-methylgalactitol derivative, not being removed, and the D-galactose to D-mannose linkage being more stable than the D-galactose-to-D-galactose linkage[9].

Partial acetolysis of the polysaccharide provided fragments that were converted into free sugars. On a paper chromatogram, they had mobilities corresponding to those of galactose, mannose, and α-D-(1→2)-linked mannobiose, mannotriose, and mannotetraose, which is consistent with consecutive (1→2)-linkages between α-D-mannopyranosyl residues.

Partial hydrolysis of the polysaccharide for 8 h at pH 1.8 and 100° provided a degraded polymer containing mannose, galactose, and glucose in the ratio of 47:15:38. Treatment with the exo-α-D-mannosidase from *Arthrobacter* sp. GJM-1 removed α-D-mannopyranosyl side-chains. Methylation–g.l.c.–m.s. indicated acetates of 2,3,4- and 3,4,6-tri-*O*-methylmannitol with a peak ratio of 3.1:1, showing a linear structure with mainly (1→6)-linkages. Another main peak corresponded to 2,3,6-tri-*O*-methylglucitol triacetate and arose from a linear glucan.

The ^{13}C-n.m.r. spectrum of the polysaccharide preparation shows a close resemblance to that obtained for the polysaccharide from *A. niger*, as would be expected from the aforementioned data. This polysaccharide had been obtained *via* an insoluble copper complex formed with Fehling solution. The structure of this galactomannan consists mainly of a mannan core having a main chain of (1→6)-linked α-D-mannopyranosyl residues, substituted at O-2 with side-chains of α-D-mannopyranosyl, *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl, and *O*-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-*O*-D-mannopyranosyl residues. The core was also substituted by side-chains of (1→5)-linked β-D-galactofuranosyl residues having an average length of 4 units. The assigned C-1 signals were: δ 108.4, 5-*O*-substituted β-D-galactofuranosyl residues; 103.6, α-D-mannopyranosyl nonreducing end-groups; and 99.9, 2,6-di-*O*-substituted α-D-mannopyranosyl residues. A minor signal at δ 109.2 was attributed to C-1 of a β-D-galactofuranosyl linked to an α-D-mannopyranosyl residues. A recent ^{13}C-n.m.r. study[22] of disaccharides consisting of a β-D-galactofuranosyl residue linked to methyl α-D-mannopyranoside showed that the C-1′ shift is influenced by the position of the glycosyl residue, being δ 109.7 for a (1→6)-, 107.7 for a (1→2)-, and 106.5 for a (1→3)-linkage. Thus, the signal at δ 109.2 arose from a (1→6)-linkage.

The ^{13}C-n.m.r. spectra of the *A. fumigatus* polysaccharides resembled that of the polysaccharide of 10-day-old mycelia, except that an additional, broad C-1 signal was present at δ 101.5. In view of the detection of 2,3,4,6-tetra-*O*-, 2,3,6-tri-*O*-, and 2,3-di-*O*-methylglucitol acetates in the methylation analysis, this signal appears to arise from a structure resembling glycogen, as it was degraded to D-glucose with amyloglucosidase and gave a red-brown coloration with iodine. Thus, it differs from the nigeran of *A. niger*, the ^{13}C-n.m.r. spectrum[9] of which showed two C-1 signals at δ 101.3 and 101.5.

Polysaccharide from the mycelium of a 10-day culture. — The polysaccharide isolated from a 10-day-old culture of *A. fumigatus* (see Table I) in a 0.8% yield had $[\alpha]_D^{25}$ 0° (*c* 0.3, water). As shown by its composition, the proportion of D-galactose decreased with duration of culture time, in agreement with the results obtained by Johnston[23] for *A. niger*. Also, the proportion of D-glucose, as represented by glycogen, decreased.

Methylation, followed by hydrolysis, sodium borohydride reduction, and acetylation gave *O*-methylalditol acetates (Table II), indicating that the isolated fragments were similar to those obtained from the 5-day-old polysaccharides, except that the ratio of methylated sugars was different.

The D-galactosyl units were partly removed with acid to give a polysaccharide containing D-mannose, D-galactose, and D-glucose in the ratio of 5:4:6. Methylation data (Table II) for this degraded polysaccharide indicate that, as with the 5-day-old polysaccharide, all D-galactofuranosyl residues (represented by the 2,3,5,6-tetra-*O*-methylgalactitol derivative) were not removed.

Partial hydrolysis of the polysaccharide gave a product showing a ^{13}C-n.m.r. spectrum devoid of the signal at δ 108.4, but with signals at δ 109.4 and 64.5, which

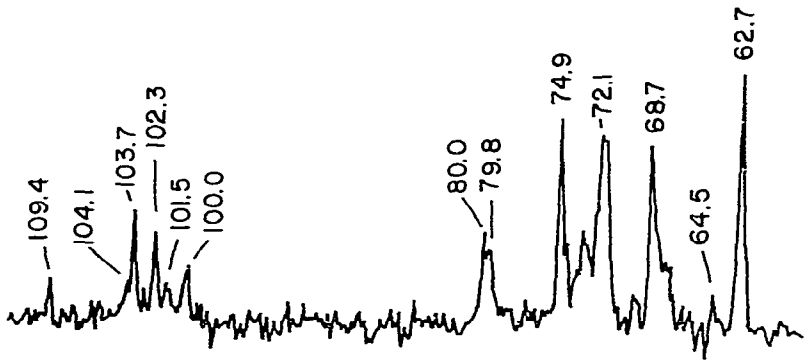

Fig. 1. ^{13}C-N.m.r. spectrum of polysaccharides derived by partial hydrolysis of polysaccharides isolated from 10-day-old mycelium of *A. fumigatus*.

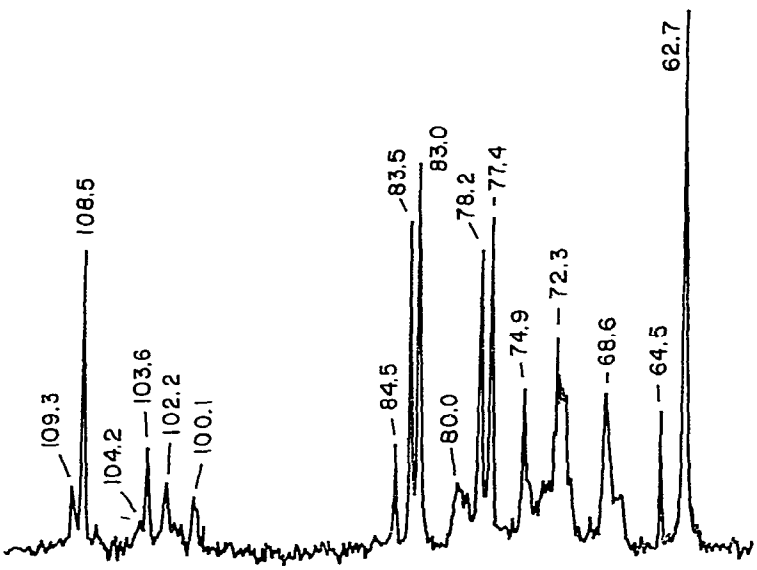

Fig. 2. ^{13}C-N.m.r. spectrum of polysaccharides isolated from 10-day-old mycelium of *A. fumigatus*.

arise, respectively, from C-1 and C-6 of single-residue D-galactofuranosyl side-chains having a (1→6)-linkage to the adjacent α-D-mannopyranosyl unit more stable than the other furanosyl linkages (Fig. 1).

Partial acetolysis of the polysaccharide gave galactose, mannose, and α-D-(1→2)-linked mannobiose, mannotriose, and mannotetraose, as with the 5-day polysaccharide.

The ^{13}C-n.m.r. spectrum of the polysaccharide (Fig. 2) differs from that of the 5-day-old preparation by the presence of a smaller C-1 signal at δ 101.5, which corresponds to glycogen (characterized by methylation data, enzymic degradation with amyloglucosidase, and a red-brown coloration with iodine). The methylation studies did not show a stepwise removal of galactosyl units with time[24].

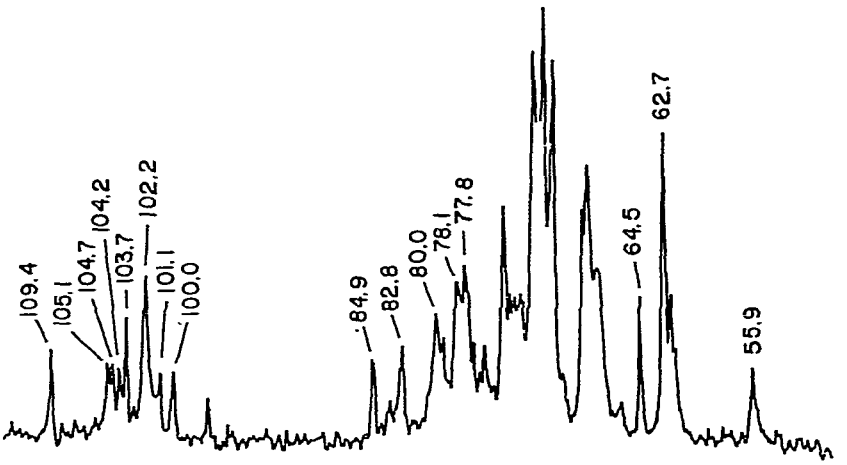

Fig. 3. ^{13}C-N.m.r. spectrum of polysaccharides isolated from conidia of *A. fumigatus*.

Polysaccharide from conidia. — Extraction of isolated conidia with aqueous hot alkali gave a polysaccharide (see Table I) in 2% yield having $[\alpha]_D^{25}$ +26° (*c* 0.3, water). The ^{13}C-n.m.r. spectrum (Fig. 3) was considerably different from that of mycelial preparations as it lacked, at δ 108.4, a C-1 signal corresponding to (1→5)-linked β-D-galactofuranosyl residues, and showed only signals at δ 109.4 and 64.5 corresponding to C-1 and C-6, respectively, of single-residue β-D-galactofuranosyl side-chains (1→6)-linked to α-D-mannopyranosyl units. Signals were detected at δ 104.2, 104.7, and 105.1, which were not present in the spectra of the mycelial polysaccharides. Another characteristic of the spectrum of the conidial polysaccharide was a signal at δ 55.9 that appears to correspond to C-2 of 2-amino-2-deoxyhexosyl residues. Examination of the hydrolyzate on an amino acid analyzer revealed the presence of 1–2% of 2-amino-2-deoxyglucose.

Methylation analysis of the conidial polysaccharides (Table II) gave *O*-methylalditol acetates that indicated branched structures containing D-galactofuranosyl and D-mannopyranosyl nonreducing end-groups, 2-*O*- and 6-*O*-substituted D-mannopyranosyl, and 2,6-di-*O*-substituted mannopyranosyl residues. Absent were fragments corresponding to (1→5)-linked β-D-galactofuranosyl residues, in agreement with the ^{13}C-n.m.r. data, but the characterization of 2,3,5-tri-*O*-methylgalactitol acetate (2%) showed that 6-*O*-substituted D-galactofuranosyl units were present. A β-Gal*f*-(1→6)-β-Gal*f* structure would give a C-1′ signal[22,25] at δ 109.5, but the small proportion of this structure would preclude a significant contribution to the C-1 signal at δ 109.4 (Fig. 3).

Other structures observed, differing from those present in mycelial polysaccharides, are D-galactopyranosyl nonreducing end-groups and a highly branched (1→4), (1→6)-linked glucan, not degraded to D-glucose by the action of amyloglucosidase.

The conidial polysaccharides were partly acetolyzed, and the resulting free sugars examined on a paper chromatogram. Spots having mobilities and color

TABLE III

FATTY ACIDS FROM LIPID FRACTIONS OF WHOLE CELLS AND CELL WALLS OF *A. fumigatus* (%)[a]

Fatty-acid	*5-Day-old mycelium*		*10-Day-old mycelium*		*Conidia*	
Fraction	*WC*	*CW*	*WC*	*CW*	*WC*	*CW*
Lipid Fraction I						
C_{16}	17.1	27	16.2	25.2	29.4	22.1
C_{18}	28.4	37.2	22	32.2	16	19
$C_{18:1}$	25.1	28.5	25.6	34.8	40	33.1
$C_{18:2}$	29.2	7	33	7.5	14.5	20.7
Lipid Fraction II						
C_{16}	21	48.7	17.5	54.3	15.8	57.3
C_{18}	32.1	42	22.7	37.3	28.4	42.6
$C_{18:1}$	21.8	6.4	26.7	6.2	27.7	tr[b]
$C_{18:2}$	25	2.1	33	2	27.7	tr[b]

[a]Lipid Fractions I and II, according to Bartnicki-Garcia and Nickerson[20]. Abbreviations: WC, whole cells; and CW, cell walls. [b]Trace.

TABLE IV

CONTENTS OF PROTEIN AND AMINO ACIDS OF WHOLE CELLS AND CELL WALLS FROM MYCELIA AND CONIDIA OF *A. fumigatus*

	Mycelium				*Conidia*	
	Whole cells		*Cell walls*		*Whole cells*	*Walls*
	5-Day-old	*10-Day-old*	*5-Day-old*	*10-Day-old*		
Protein[a]	336	282	278	235	173	53
Amino acids[b]						
Lysine	148	113	116	78	79	20
Arginine	131	96	90	59	50	15
Histidine	15	9	34	24	3	9
Aspartic acid	258	207	214	159	165	43
Threonine	128	101	110	82	80	25
Serine	158	121	126	97	107	30
Glutamic acid	325	256	199	145	154	37
Proline	133	112	94	65	89	27
Glycine	268	232	184	150	145	44
Alanine	241	197	186	136	128	38
Valine	152	140	149	124	93	34
Methionine	21	17	7	5	[c]	
Cysteine	40	49	20	16	17	8
Isoleucine	100	78	97	64	65	22
Leucine	170	135	166	116	101	29
Tyrosine	203	170	207	253	73	31
Phenylalanine	129	164	135	178	41	33

[a]Values are given in ‰ of whole cells or cell walls. [b]Values are given in μmol/g of whole cells or cell walls. [c]Trace.

reactions corresponding to galactose, mannose, and an α-D-(1→2)-linked mannopyranosyl disaccharide were observed. Thus, it appears that the polysaccharide has sequences of (1→2)-linked α-D-mannopyranosyl residues much shorter than those of the mycelial product.

Lipid fractions of A. fumigatus. — The proportions of fatty acids in the lipid fractions of whole cells and cell walls from *A. fumigatus* are shown in Table III. Little variation was observed between fatty acid contents in 5- or 10-day-old mycelial whole cells. The conidia contained different proportions of fatty acids in lipid Fraction I, but lipid Fraction II resembled that from the mycelium. The mycelial walls had much less $C_{18:2}$ in Fraction I and less $C_{18:2}$ in Fraction II, when compared to whole cells. $C_{18:1}$ and $C_{18:2}$ fatty acids were detected in trace amounts in lipid Fraction II from the conidial cell wall, but the $C_{18:2}$ fatty acid content was high (20.7%) in Fraction I, which differed from the mycelial wall.

Protein and amino acid contents. — The distribution of protein and amino acids in various cell types of *A. fumigatus* is shown in Table IV. Conidia had considerably less total protein than hyphae of whole cells or cell walls. Prolonged incubation (10 days) was accompanied by a decrease in the protein content of the mycelium. Histidine and aromatic amino acids seemed to be preferentially distributed among cell-wall proteins of hyphae. No such distribution is suggested for the conidial proteins.

DISCUSSION

The mycelia of *A. niger* contain nigeran, in contrast to those of *A. fumigatus*, the D-glucan component of which is glycogen, as shown by methylation data, treatment with amyloglucosidase, and a red-brown coloration produced with an iodine solution. In neutralized alkali extracts of hyphae of *A. fumigatus*, glycogen is present as a contaminant of the D-galacto-D-mannan[6]. In the present work, the polysaccharide mixtures obtained from mycelia and conidia were structurally characterized, and the types of galactomannan and glucans compared.

The 5-day-old mycelium forms a D-galacto-D-mannan consisting of a main chain containing (1→6)-linked α-D-mannopyranosyl residues substituted at O-2 by side chains containing one to three α-D-mannopyranosyl residues (1→2)-linked. β-D-Galactofuranosyl residues are present as side chains of ~6 units that are (1→5)-interlinked. The β-D-galactofuranosyl units are (1→6)-linked to the D-mannan core. Such a complex D-galacto-D-mannan is very similar to a polysaccharide isolated from *A. niger* described earlier[9]. A partial description of the structure of an *A. fumigatus* D-galacto-D-mannan was presented by Azuma *et al.*[6], but the configuration of the residues and the structure of the β-D-galactofuranosyl side-chains were not determined. These authors proposed that the (1→6)-linked D-mannose residues were located in side chains. Side chains containing (1→4)-linked β-D-galactopyranosyl units, as suggested by Bardalaye and Nordin[26], have not been confirmed by our results with either mycelial polysaccharides of *A. niger*[9] or *A. fumigatus*. Only β-D-galactofuranosyl units were detected in these polysaccharides.

Although prolonged (10 days) incubation of *A. fumigatus* resulted in polysaccharides containing a proportion of D-galactose lower than that observed for the polysaccharides from the 5-day-old incubation, this observation could not be correlated with a preferential removal of β-D-galactofuranosyl side-chains, as the methylation data (Table II) showed that their length was unchanged. However, reduction of the glycogen content occurred on incubation.

Antigens have been prepared from *A. fumigatus*, both in a crude form[27–31], or as a purified D-galacto-D-mannan–protein complex[32] and derived D-galacto-D-mannan. It appears that each of these preparation would contain a D-galacto-D-mannan with long side-chains of (1→5)-linked β-D-galactofuranosyl residues, as described above.

The presence of other chemical structures in the conidial polysaccharide was demonstrated by its ^{13}C-n.m.r. spectrum (Fig. 3), which clearly differs from that of the mycelial polysaccharide (Fig. 1). The most significant difference is the presence of single-unit side-chains of (1→6)-linked β-D-galactofuranosyl residues, likely to α-D-mannopyranosyl residues of the main chain. Such side-chains have been previously observed in D-galacto-D-mannans of dermatophytes[33], the β-D configuration and the (1→6)-interresidue linkage being confirmed for the D-galacto-D-mannan of *Trichophyton interdigitale*[22]. It is conceivable that various structures of the mycelial and conidial D-galacto-D-mannans may also correspond to various antigens in *A. fumigatus*. A D-galacto-D-mannan from *A. fumigatus* did not cross-react with rabbit sera raised against *H. capsulatum* and *P. brasiliensis*[34], suggesting that there are at least two types of serologically-active D-galacto-D-mannans in fungi.

Several minor chemical structures have been observed for the conidial, but not for the mycelial polysaccharide. These are D-galactopyranosyl nonreducing end-groups, and 6-*O*-substituted β-D-galactofuranosyl and 2-amino-2-deoxyglucosyl residues.

The main constituents of the cell wall of *A. fumigatus* were determined by Hearn and Mackenzie[3]. Present results with another strain of *A. fumigatus* (NCPF 2109) differ from that communication in two respects: The D-glucose component of the cell-wall polysaccharides discussed here represented more than 60% of the neutral carbohydrate fraction, whereas, in the former study[3], it accounted for 29% of the corresponding fraction, D-galactose being the preponderant sugar (52%). Also, the protein content reported before[3] for *A. fumigatus* cell-wall was 10% by weight, whereas the mycelial walls of the present study contain 23–28% of protein. However, an isolated conidial, cell-wall preparation of the same strain of *A. fumigatus* had only 5% of protein. Azuma *et al.*[6] extracted a peptidogalactomannan from ground mycelium and conidia of *A. fumigatus* and determined the amino acid composition of the fraction precipitated by 66% acetone. Serine and threonine were the preponderant amino acids, as observed for other isolated fungal glycopeptides[35,36]. In contrast, the mycelial, cell-wall proteins of *A. fumigatus* showed aspartic and glutamic acids, tyrosine, alanine, and glycine as preponderant amino acids. It is clear, then, that other structural proteins are present in the cell wall in addition to the peptidogalactomannan antigen.

Analysis of the fatty acids of *A. fumigatus* whole cells and walls showed the presence of C_{16}, C_{18}, $C_{18:1}$, and $C_{18:2}$ fatty acids, as in *Sporothrix schenckii* cell-walls[19]. The $C_{18:3}$ fatty acid, detected in yeast forms of *S. schenckii*, was not present in *A. fumigatus* lipid-fractions. The $C_{18:2}$ fatty acid was preponderant in whole cells of *A. fumigatus*, but not in the mycelial, cell-wall fraction, where it was a minor component. The $C_{18:2}$ fatty acid was, however, present in the proportion of 21% in lipid Fraction I of the conidial cell-wall. No other significant differences were observed for the fatty acid contents of lipid fractions from mycelia and conidia.

Antigenic preparations from *A. fumigatus* were reactive with sera from human patients with both aspergilloma and allergic bronchopulmonary aspergillosis, although the latter sera had to be concentrated 6-fold because of the low titer[37]. It is unclear whether differences in cell-wall structures (mainly low protein-content and different polysaccharide structures) in conidia as compared to the mycelium are associated with the clinical evolution of aspergillosis.

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REFERENCES

1 R. M. Coleman and L. Kaufman, *Appl. Microbiol.*, 23 (1972) 301–308.
2 T. H. Dee, *J. Clin. Microbiol.*, 2 (1975) 482–485.
3 V. M. Hearn and D. W. R. Mackenzie, *J. Gen. Microbiol.*, 112 (1979) 35–44.
4 J. L. Longbottom, J. Pepys, and F. T. Clive, *Lancet*, (1964) 588–589.
5 I. Azuma, H. Kimura, F. Hirao, E. Tsubura, and Y. Yamamura, *Jpn. J. Med. Mycol.*, 8 (1967) 210–220.
6 I. Azuma, H. Kimura, F. Hirao, E. Tsubura, Y. Yamamura, and A. Misaki, *Jpn. J. Microbiol.*, 15 (1971) 237–246.
7 K. Yokota, T. Sakaguchi, and M. Suzuki, *Jpn. J. Med. Mycol.*, 13 (1972) 203–206.
8 S. Suzuki and N. Takeda, *Carbohydr. Res.*, 40 (1975) 193–197.
9 E. Barreto-Bergter, L. R. Travassos, and P. A. J. Gorin, *Carbohydr. Res.*, 86 (1980) 273–285.
10 J. S. Sawardeker, J. H. Sloneker, and A. Jeanes, *Anal. Chem.*, 37 (1965) 1602–1604.
11 W. N. Haworth, *J. Chem. Soc.*, 107 (1915) 8–16.
12 R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, 67 (1955) 32.
13 H. Björndal, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, 5 (1967) 433–440.
14 Y.-C. Lee and C. E. Ballou, *Biochemistry*, 4 (1965) 257–264.
15 L. Hough and J. K. N. Jones, *Carbohydr. Chem.*, 1 (1962) 21–31.
16 S. M. Partridge, *Nature (London)*, 158 (1946) 270–271.
17 G. H. Jones and C. E. Ballou, *J. Biol. Chem.*, 244 (1969) 1043–1051.
18 P. A. J. Gorin, R. H. Haskins, L. R. Travassos, and L. Mendonça-Previato, *Carbohydr. Res.*, 55 (1977) 21–33.
19 J. O. Previato, P. A. J. Gorin, and L. R. Travassos, *Exp. Mycol.*, 3 (1979) 83–91.
20 S. Bartnicki-Garcia and W. J. Nickerson, *Biochim. Biophys. Acta*, 58 (1962) 102–119.
21 A. P. Tulloch, *Lipids*, 9 (1974) 664–668.
22 E. Barreto-Bergter and P. A. J. Gorin, *ACS Symp. Ser.*, 150 (1981) 149–159.
23 I. R. Johnston, *Biochem. J.*, 96 (1965) 651–658.
24 M. Rietschel-Berst and J. E. Gander, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 32 (1973) 528.

25 L. MENDONÇA-PREVIATO, P. A. J. GORIN, AND L. R. TRAVASSOS, *Infect. Immun.*, 29 (1980) 934–939.
26 P. C. BARDALAYE AND J. H. NORDIN, *J. Biol. Chem.*, 252 (1977) 2584–2591.
27 M. SUZUKI, S. SUZUKI, K. YOKOTA, AND O. SAKAGUCHI, *Nippon Saikingaku Zasshi*, 21 (1966) 114–119.
28 M. SUZUKI AND Y. HAYASHI, *Jpn. J. Microbiol.*, 19 (1975) 355–362.
29 M. SUZUKI AND Y. HAYASHI, *Nippon Saikingaku Zasshi*, 30 (1975) 583–588.
30 P. TRAN VAN KY, J. BIGUET, AND T. VAUCELLE, *Rev. Immunol. Ther. Antimicrob.*, 32 (1968) 37–52.
31 I. WADA, *Nippon Saikingaku Zasshi*, 15 (1966) 528–530.
32 O. SAKAGUCHI, K. YOKOTA, AND M. SUZUKI, *J. Pharm. Sci.*, 87 (1967) 82–87.
33 C. T. BISHOP, M. B. PERRY, F. BLANK, AND F. P. COOPER, *Can. J. Chem.*, 40 (1965) 30–39.
34 I. AZUMA, F. KANETSUNA, Y. TANAKA, Y. YAMAMURA, AND L. M. CARBONELL, *Mycopathol. Mycol. Appl.*, 54 (1974) 111–125.
35 K. O. LLOYD AND M. A. BITOON, *J. Immunol.*, 107 (1971) 663–671.
36 L. R. TRAVASSOS, W. SOUZA, L. MENDONÇA-PREVIATO, AND K. O. LLOYD, *Exp. Mycol.*, 1 (1977) 293–305.
37 V. M. HEARN AND D. W. R. MACKENZIE, *Med. Mycol., Proc. Mycol. Symp., Int. Congr. Microbiol., XIIth*, Suppl. 8 (1978) 173–181.