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CHEMICAL STRUCTURE OF THE D-GALACTO-D-MANNAN COMPONENT FROM HYPHAE OF *Aspergillus niger* AND OTHER *Aspergillus spp.**

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ABSTRACT

The polysaccharides obtained by alkaline extraction of hyphae of *Aspergillus niger* 2022 contain D-mannosyl, D-galactosyl, and D-glucosyl units, the proportion of D-galactose decreasing and that of D-mannose increasing with age of culture. The polysaccharide of 5-day cultures contains D-galacto-D-mannans with a range of chemical structures, Fehling precipitation providing polysaccharides of sugar composition which varied from one preparation to the other. This step removed most of the α-D-glucan present and analysis of the D-galacto-D-mannan-rich polysaccharide showed it to contain a core with a main chain of (1→6)-linked α-D-mannopyranosyl units substituted in the 2-position with side chains of α-D-mannopyranosyl, *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl, and *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl units. The methylation and ^{13}C-n.m.r. data indicated side chains of (1→5)-linked β-D-galactofuranosyl residues having an average length of ~4 units and linked to the mannan core at OH-6. Polysaccharides of *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus flavus*, and *Aspergillus nidulans*, prepared by alkaline extraction of hyphae, and precipitated by Cetavlon in the presence of borate have interrelated chemical structures.

INTRODUCTION

The cell wall of fungi consists mainly of polysaccharides. In *Aspergillus niger*, proportions as high as 9–13% of hexosamine and 73–83% of neutral carbohydrate have been reported. Glucans (73–91%) are the main constituents of the neutral carbohydrate fraction with lesser amounts of polysaccharides composed of galactose (6–22%) and mannose (3–5%) (ref. 1). Arabinose[1], glucosamine, and galactos-

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amine[2] were also detected in hydrolyzates. The predominant glucan is nigeran[1], which consists of alternating (1→3)- and (1→4)-linked α-D-glucopyranosyl units[3,4]. Pseudonigeran, an α-D-(1→3)-linked D-glucopyranan, may also be present[5,6]. β-D-Linked D-glucans may also occur, according to ^{1}H-n.m.r. data of the per-*O*-methyl derivative of a glucan extract[8]. The chitin component of the cell wall[9,10] has been reported to be associated with D-glucan[8].

Hyphae of *A. niger* contain 0.6–0.8% of a water-insoluble polysaccharide, the structure of which consists principally of (1→4)-linked α-D-galactopyranosyl units and (1→4)-linked 2-amino-2-deoxy-D-galactopyranosyl units, some of which are *N*-acetylated[11]. The presence[12] of a pure, water-insoluble galactan in *A. niger* has not since been substantiated.

Galactomannans[13] that are immunologically active[14,15] have been identified. They show cross reactions with anti-sera that are apparently specific for D-galacto-furanosyl units. An aqueous alkali extract of *A. niger* cell-wall was examined by Bardalaye and Nordin[16], who proposed a general structure with a main chain of (1→2)- and (1→6)-linked (minor) α-D-mannopyranosyl units, and side chains of β-D-Gal*f*-(1→4)-D-Gal*p*-(1→4)-D-Gal*p*-(1→4)-D-Gal*p*- units and other side-chains with one less D-galactopyranosyl unit.

The present paper describes a study of related, alkali-soluble components from *A. niger* that can be purified by means of the water-insoluble copper complex formed with Fehling solution. The sugar composition of the alkali extract varies with age of culture, as would be anticipated from the investigations of Johnston[1]. It was compared with alkali-soluble polysaccharides isolated from hyphae of other species of *Aspergillus* by use of ^{13}C-n.m.r. spectroscopy.

EXPERIMENTAL

Culture of microorganisms. — The following strains were supplied by the Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine, U.K.: *Aspergillus niger* 2022, *Aspergillus fumigatus* 2078, *Aspergillus flavus* 2008, *Aspergillus terreus* 2026, and *Aspergillus nidulans* 2182. The fungi were maintained in a medium containing per liter: peptone (Difco) 10 g, yeast extract (Difco) 5 g, agar (Difco) 20 g, and D-glucose 40 g. Following inoculation of plugs, they were incubated for 72 h at 25°, and then kept at 4° under a layer of mineral oil, transfers being made every 6 months. Hyphae were grown at pH 6.5 on a medium (*A*) containing per liter: D-glucose 20 g, peptone 10 g, and low-molecular-weight components of yeast extract obtained by dialysis 5 g.

The cells obtained by growth on solid Sabouraud medium were initially inoculated into 500-mL Erlenmeyer flasks containing medium *A* (200 mL). The cells were incubated at 25° or 37° with shaking for 3–4 days, and then transferred to 3 L of the same medium distributed equally between Fernbach flasks (2.8 L). The incubation was carried out for 5 to 10 days at 25° for cells of *A. niger* and for 7 days at 25° and 37° for those of other *Aspergillus* spp.

Isolation of hyphae, and extraction and purification of polysaccharide. — The cells were isolated by centrifugation, dispersed in water, and recentrifuged, the process being repeated 3 times, and the washed cells were lyophilized. In a typical 10-day experiment, *A. niger* gave 9.1 g of dry cells per liter of medium.

Dry *A. niger* cells (80 g) were extracted for 6 h in 6% aqueous potassium hydroxide (4 L) at 100°. The suspension was neutralized with acetic acid, and then centrifuged, and the supernatant evaporated to a small volume. An excess of ethanol was added, and the resulting precipitate was filtered off, washed with ethanol, and dried to provide 10 g of crude polysaccharide. This was dissolved in water, the solution passed through columns of Amberlite IR-120 (H^+) and Dowex 1-X8 (OAc^-) ion-exchange resins, and the eluate evaporated to a small volume. The polysaccharide was obtained by precipitation with ethanol (yield, 1 g), $[\alpha]_D^{25}$ +53° (*c* 0.5, water). The insoluble copper complex was isolated following dissolution of the polysaccharide in a small quantity of water and addition of Fehling solution[17]. The regenerated polysaccharide (50 mg) had $[\alpha]_D^{25}$ +4° (*c* 0.2, water).

Hyphae of other *Aspergillus* spp. were extracted similarly except that 2% aqueous potassium hydroxide was used. Crude polysaccharides were purified *via* precipitates formed with hexadecatrimethylammonium bromide (Cetavlon) in the presence of sodium borate[18] at pH 8.8.

Characterization and quantitative determination of component sugars of polysaccharides. — Polysaccharide preparations were hydrolyzed with 0.5M sulfuric acid for 18 h at 100°. Hydrolyzates were neutralized ($BaCO_3$), filtered, and evaporated to residues containing monosaccharides, which were reduced with sodium borohydride, and the resulting alditols converted to their acetates. These were characterized and quantitatively determined relative to each other, by g.l.c.[19]

Methylation of polysaccharides. — Polysaccharides were methylated by the method of Haworth[20], the partially methylated products being isolated by dialysis and evaporation, and then completely methylated by the procedure of Kuhn *et al.*[21]. Each per-*O*-methyl derivative was boiled under reflux in methanol containing 3% of hydrogen chloride for 18 h, the acid was neutralized (Ag_2CO_3), the suspensions filtered, and the filtrate evaporated to a syrup. This was completely hydrolyzed with 10% aqueous sulfuric acid for 18 h at 100°, the solution neutralized ($BaCO_3$), filtered, and evaporated. The product was reduced with sodium borohydride and then acetylated. The partially methylated alditol acetates were examined by g.l.c.–m.s., and characterized by their retention times and mass patterns obtained by electron impact[22]. The column packing was 3% of ECNSS-M on Chromosorb W (80–100 mesh), programmed from 120° to 170° at 4°/min. In order to separate and estimate the acetates of 2,3,4-tri-*O*-methylmannitol and 2,3,6-trimethylgalactitol, 3% of neopentylglycol sebacate was used as the liquid phase, with a program from 140° to 190° at 4°/min.

Partial acetolysis of D-*galacto*-D-*mannan.* — The partial acetolysis was performed according to the procedure of Lee and Ballou[23], the products were *O*-deacetylated, and the de-ionized sugars examined on paper chromatograms in 2:1:1 (v/v)

1-butanol–ethanol–water with *p*-anisidine hydrochloride[24] and ammoniacal silver nitrate as sprays[25]. The mobility of the components was compared to those of 2-*O*-α-D-mannopyranosyl-D-mannose, and α-D-(1→2)-linked mannotriose and mannotetraose.

^{13}C-N.m.r. spectroscopy. — ^{13}C-N.m.r. data were obtained as previously described[26], at 70°, using polysaccharide samples of 50–200 mg dissolved in D_2O deuterium oxide (2 mL) in a 12-mm diameter tube. For quantities of 10–50 mg, a coaxial cylindrical cell (1-mL capacity) was used. Chemical shifts are expressed as δ_c, relative to an external standard of tetramethylsilane, the resonance of which was determined in a separate experiment.

Partial hydrolysis of D-*galacto*-D-*mannans, and enzymolysis of product.* — The sample of D-galacto-D-mannans (5 mg) was dissolved in water (5 mL), which was acidified to pH 2 or pH 1.8, and heated at 100° for the required time. The degraded polysaccharide was recovered following dialysis and lyophilization.

The polysaccharide (3 mg), obtained after partial hydrolysis at pH 2 for 18 h, was dissolved in water (2 mL) and treated with crude exo-α-D-mannosidase[27] (5 mg) from *Arthrobacter* sp. GJM-1. The reaction was terminated by heating after 4 h, when enzymolysis of side chains was complete, and the mixture dialyzed. One portion of the resulting polymer was methylated and the resulting *O*-methylalditol acetates identified. Another sample was examined, as a solution in deuterium oxide at 70°, by (^{1}H-n.m.r.) spectroscopy[28].

RESULTS

A polysaccharide was extracted from intact hyphae of *A. niger* 2022, grown for 10 days at 25°, with hot, 6% aqueous potassium hydroxide at 100°. Under these conditions, the protein component was hydrolyzed and any residual nucleic acids were removed by passage through a column of mixed ion-exchange resins. Precipitation by ethanol from the de-ionized solution provided a polysaccharide having $[\alpha]_D^{25}$ +53°, in 1% yield (Fraction A), and this contained mannose, galactose, and glucose in a ratio of 7:7:11. This material was fractionated *via* the insoluble copper complex formed with Fehling solution, and the resulting polysaccharide (Fraction B) had $[\alpha]_D^{25}$ +4° and contained residues of the aforementioned sugars in a ratio of 48:33:18. The polysaccharide of the mother liquor contained the same monosaccharide components in a ratio of 11:14:25. In another Fehling precipitation experiment, carried out on Fraction A, the insoluble component contained mannose, galactose, and glucose in a ratio of 65:21:12, a value that was not changed by a subsequent precipitation step.

A polysaccharide of the Fraction A type, isolated from hyphae grown for only 5 days, gave on hydrolysis mannose, galactose, and glucose in a ratio of 35:48:18. The Fehling-insoluble fraction was isolated and the resulting polysaccharide gave, on hydrolysis, a sugar ratio of 61:29:10.

The chemical structure of Fraction B (sugar ratio 48:33:18), from hyphae of

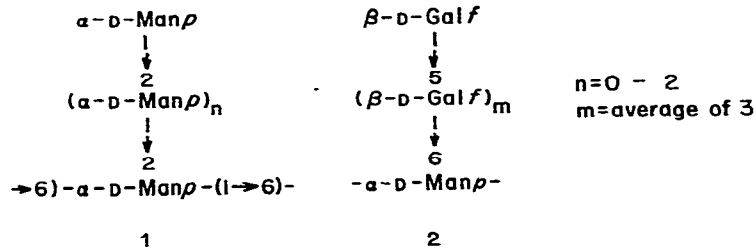
a 10-day culture, was investigated. Methylation, followed by hydrolysis and conversion of *O*-methylalditol acetates, gave derivatives of 2,3,4,6-tetra-*O*-methylmannitol, 2,3,5,6-tetra-*O*-methylgalactitol, 3,4,6-tri-*O*-methylmannitol, 2,3,6-tri-*O*-methylgalactitol, 2,3,4-tri-*O*-methylmannitol, and 3,4-di-*O*-methylmannitol in the ratio 23:14:12:36:5:9 (relative areas of peaks, as detected by g.l.c.). The low proportion of di-*O*-methylmannitol acetate is likely due to preferential degradation of the di-*O*-methylmannose during the acid hydrolysis step. The derivatives were characterized by their retention times and mass spectrometric breakdown patterns[22]. Under the conditions used, the small peak of 2,4,6-tri-*O*-methylglucitol acetate, derived from nigeran, is hidden under that of 3,4,6-tri-*O*-methylmannitol acetate. The 2,3,4,6-tetra-*O*-methylhexitol peak could also contain some glucitol derivative in addition to mannitol. The highly-branched structure therefore contains galactofuranosyl and mannopyranosyl (and perhaps glucopyranosyl) nonreducing end-units, 2-*O*- and 6-*O*-substituted mannopyranosyl units, 4-*O*-substituted galactopyranosyl or 5-*O*-substituted galactofuranosyl units (or both), and 2,6-di-*O*-substituted mannopyranosyl units.

Galactosyl units were partly removed by hydrolysis of Fraction B for 16 h at 100° at pH 1.8, providing a degraded polysaccharide that was isolated, after dialysis to remove low-molecular-weight degradation products. This polysaccharide gave, after partial hydrolysis, mannose, galactose, and glucose in a ratio of 77:10:12. When the hydrolysis was performed at pH 2, ratios of 66:19:14 and 72:15:13 were obtained for polysaccharides isolated after 8- and 18-h partial hydrolysis, respectively. The latter polysaccharide was analyzed by the methylation technique and g.l.c.–m.s. Acetates of 2,3,4,6-tetra-*O*-methylmannitol, 2,3,5,6-tetra-*O*-methylgalactitol, 3,4,6-tri-*O*-methylmannitol, 2,3,6-tri-*O*-methylgalactitol, 2,3,4-tri-*O*-methylmannitol and 3,4-di-*O*-methylmannitol were detected in the ratio 21:15:24:4:15:18. Thus, under the partial hydrolysis conditions, all D-galactofuranosyl units, as represented by the 2,3,5,6-tetra-*O*-methylgalactitol derivative, were not removed.

The partially acid-degraded polysaccharide was treated with exo-α-D-mannosidase from *Arthrobacter* sp. GJM-1, which removed α-D-mannopyranosyl side-chains. Monosaccharide fragments were removed by dialysis, and the polymeric residue was examined by ^{1}H-n.m.r. spectroscopy. The H-1 region of the spectrum contained a single signal at δ 5.40, corresponding to 6-*O*-substituted α-D-mannopyranosyl units[28]. Methylation–g.l.c.–m.s. gave acetates of 2,3,4- and 3,4,6-tri-*O*-methylmannitol in a peak ratio of 18:5, thus showing a linear structure with (1→6) linkages and a smaller amount of (1→2) linkages.

Fraction B was partly acetolyzed and the resulting free sugars examined on a paper chromatogram. Spots were obtained with mobilities and color reactions corresponding to galactose, mannose, and α-D-(1→2)-linked mannobiose, mannotriose, and mannotetraose.

The earlier mentioned analytical data indicate that Fraction B has a structure that contains a main chain consisting principally of (1→6)-linked α-D-mannopyranosyl units, substituted in some of the 2-positions by side chains of α-D-mannopyranose,



1 2

and α-D-(1→2)-linked mannobiose or mannotriose side-chains as in structure **1**. Clearly side chains of galactosyl units are present (**2**), but it is not clear whether they are attached to the α-D-mannopyranosyl units in the side chains, main chain, or both.

In the ^{13}C-n.m.r. spectra of Fractions A and B, the C-1 regions of the spectra were essentially similar, except for signals in the Fraction A spectrum (Fig. 1, A)

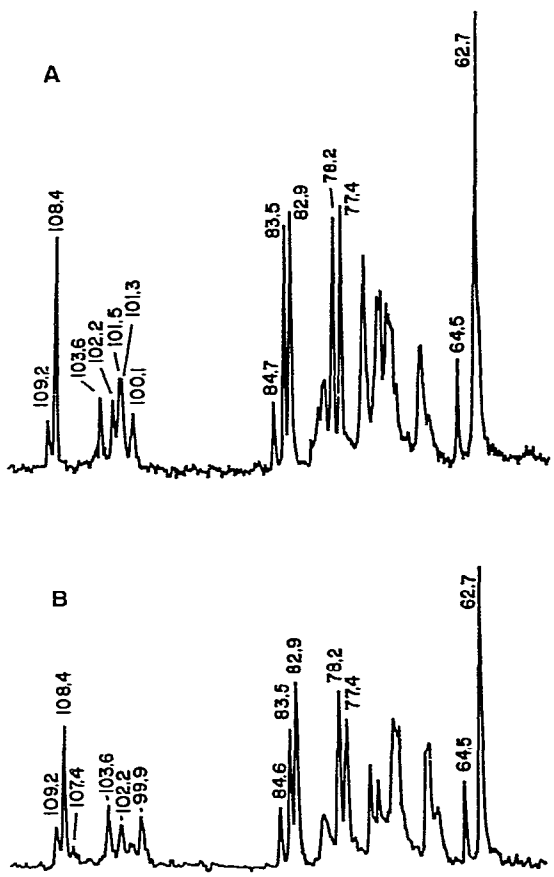
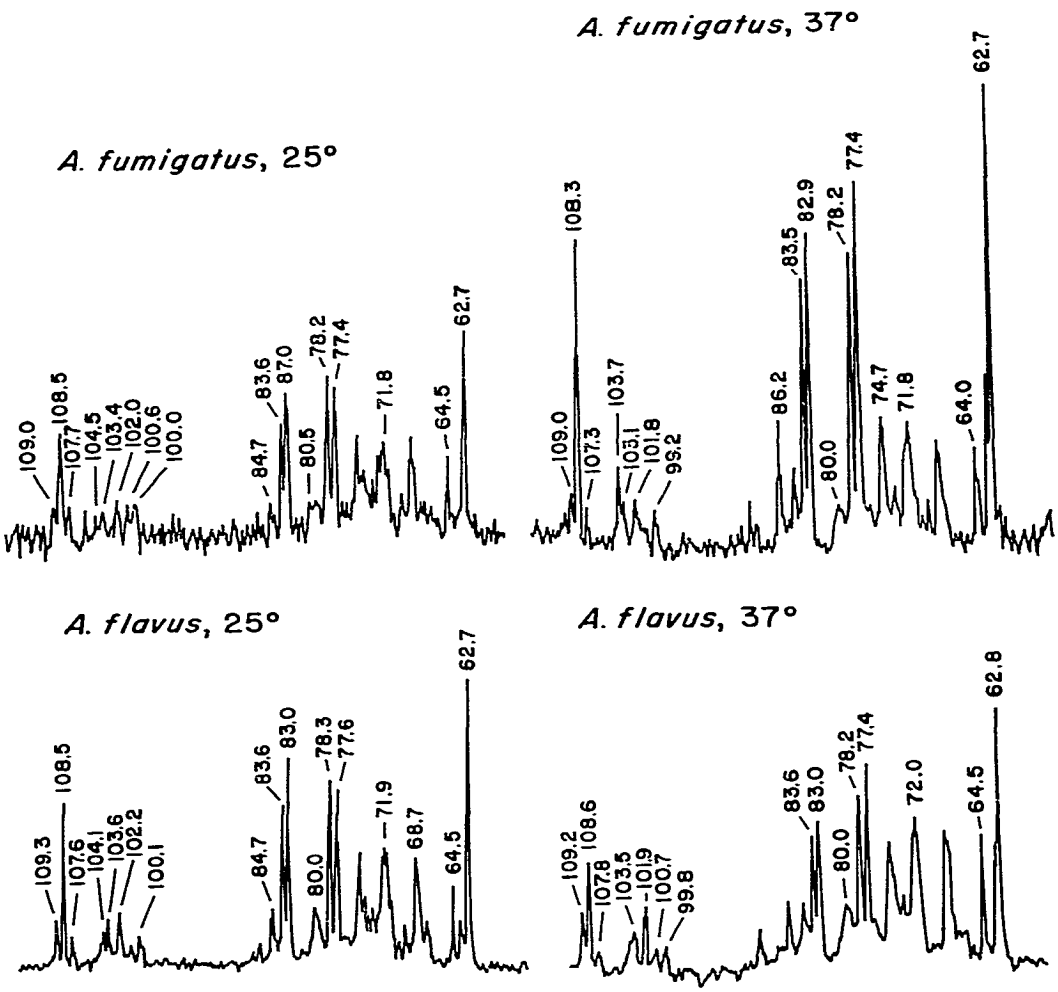


Fig. 1. ^{13}C-N.m.r. spectra of alkali extract of hyphae of *A. niger* (A) and polysaccharide isolated *via* the Fehling precipitate (B).

at δ_c 101.3 and 101.5, which correspond to the α-D-glucopyranosyl residue, probably a component of nigeran and perhaps pseudonigeran. An α-D-(1→3)-linked D-glucopyranan from *Penicillium patulum* gave a C-1 signal[29] at δ_c 101.3. The two signals were smaller in the spectrum of the Fehling-precipitated polysaccharide (Fig. 2, B). This spectrum contains signals at δ_c 108.4, 109.2 (minor), and 107.4 (trace), the major one corresponding to C-1 of the β-D-(1→5)-linked D-galactofuranosyl units[30], and the smaller ones likely corresponding to C-1 of units attached to mannopyranosyl residues[31]. The other three signals in the C-1 region would correspond, according to ^{13}C-n.m.r. data of α-D-mannans[32], to C-1 of α-D-mannopyranosyl nonreducing end units (δ_c 103.6), 2-*O*-substituted α-D-mannopyranosyl units (δ_c 102.2), and 2,6-di-*O*-substituted α-D-mannopyranosyl units (δ_c 99.9). Other prominent signals may be assigned[30] to β-D-(1→5)-linked β-D-galactofuranosyl nuclei, such as those of C-2



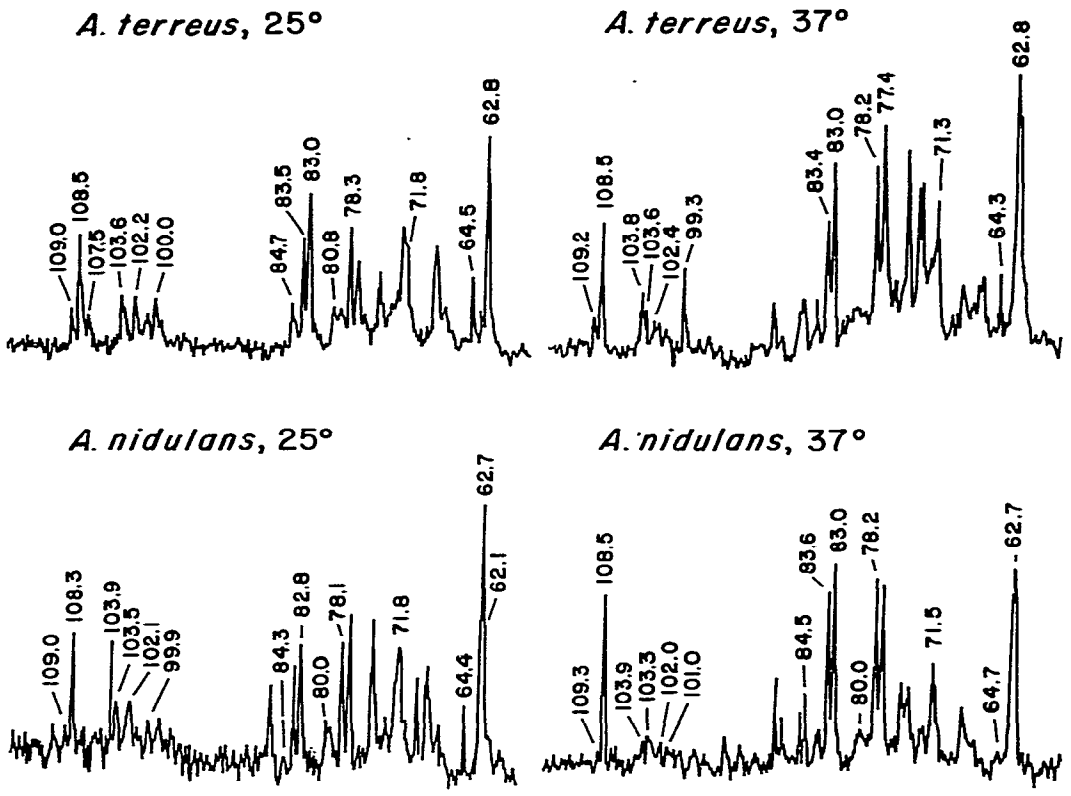


Fig. 2. ^{13}C-N.m.r. spectra of alkali extracts of hyphae of *A. fumigatus*, *A. flavus*, *A. terreus*, and *A. nidulans*, following fractionation with Cetavlon–borate.

TABLE I

PERCENTAGE COMPOSITION OF MONOSACCHARIDE COMPONENTS OF POLYSACCHARIDES OF *Aspergillus* SPP.

Polysaccharide source	*Temp. of growth (degrees)*	*Components*			$[\alpha]_D^{25}$ *(degrees)*[a]
		D-*Mannose*	D-*Galactose*	D-*Glucose*	
A. fumigatus	25	54	33	13	−11
A. fumigatus	37	56	31	12	−18
A. flavus	25	33	48	17	−11
A. flavus	37	47	36	18	−5
A. terreus	25	46	26	28	+2
A. terreus	37	62	32	6	0
A. nidulans	25[b]	48	43	8	+20
A. nidulans	37[c]	44	55	—	−25

[a]In water, *c* 0.2–0.5. [b]Contains 0.8% of glucosamine and 0.5% of galactosamine. [c]Contains 0.4% of glucosamine and 0.5% of galactosamine.

and C-4 (δ_c 82.9 and 83.5), C-3 (δ_c 78.2), and C-5 (δ_c 77.4). Also C-4 and C-6 signals of nonreducing end-units are present at δ_c 84.6 and 64.5, respectively.

^{13}C-N.m.r. spectra with similar signals (Fig. 2) were obtained from polysaccharides liberated from hyphae of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus nidulans*, grown at 25° and 37°. The alkali-soluble polysaccharides did not precipitate with Fehling solution and were purified *via* insoluble Cetavlon complexes formed in the presence of borate. Each of the spectra contained the three C-1 signals corresponding to α-D-mannopyranosyl units and signals at low field of β-D-galactofuranosyl residues, approximately proportional in size to the proportion of D-galactose present in the polysaccharide, which varied from 26 to 55% (Table I). Signals were also present corresponding to α-D-glucopyranosyl units. The specific rotations of the polysaccharides correspond to negative contribution from β-D-galactofuranosyl units and positive ones from α-D-mannopyranosyl and α-D-glucopyranosyl units.

DISCUSSION

The polysaccharides obtained by alkaline extraction of intact hyphae of *A. niger* 2022 appear to resemble those obtained from the cell wall[1]. Preparations obtained from 5-day cultures contained D-mannose, D-galactose, and D-glucose in a ratio of 35:48:18, as compared with 7:7:11 for polysaccharide isolated after 10 days of growth. Such ratios parallel the results of Johnston[1] who found that, in the cell wall, the proportion of D-galactose decreased and that of D-glucose increased with the age of culture. One possible explanation is that an exo-β-D-galactofuranosidase is active, such as that released into culture media by *Penicillium charlesii*[33].

The isolation of a polysaccharide containing D-galactose and D-mannose from an insoluble copper-complex formed with Fehling solution indicates that a D-galacto-D-mannan is present, since a pure D-galactan with furanosyl or pyranosyl units would not form a precipitate. Such D-galacto-D-mannan preparations contain a range of chemical structures, since treatment of a polysaccharide extract (Fraction A) with a D-mannose, D-galactose, and D-glucose ratio of 11:14:25 with Fehling solution gave rise to precipitates, in two different experiments, with different compositions having sugar ratios of 65:21:12 and 43:33:18.

The latter preparation, Fraction B, was investigated in more detail. It had a specific rotation of +4° as compared with +53° for the parent polysaccharide mixture. Thus, the removal of D-glucose-containing components lead to a reduction in specific rotation, thus indicating the presence of α-D-glucopyranose units, as present in nigeran[3,4] and pseudonigeran[5-7]. Starch-containing components were absent as shown by a negative iodine test. Also, the ^{13}C-n.m.r. spectrum of the Fehling-precipitated material contained very small signals at δ_c 101.3 and 101.5 (Fig. 1, B) with chemical shifts corresponding to those of α-D-glucopyranosyl rather than β-D-glucopyranosyl units[34]. These signals were much diminished, as compared with similar signals obtained in the spectrum of the starting material (Fig. 1, A). Nigeran appears

to be present[16], according to methylation data, in polysaccharides extracted from cell walls of *A. niger* and purified by fractionation with Bio-Gel P-150.

The D-galacto-D-mannan (sugar ratio 43:33:18), isolated *via* its copper complex was partly hydrolyzed to a polysaccharide with a much diminished proportion of D-galactosyl units. This, in turn, was degraded by an exo-α-D-mannosidase, which removed side chains giving mainly a linear α-D-mannopyranan, according to methylation data. Some other structures also arise from portions associated with unremoved D-galactofuranosyl residues. In another analysis, carried out by ^{1}H-n.m.r. spectroscopy, signals in the H-1 region of the spectrum[28] showed that 5% or less of (1→2) linkages were present. Thus, the formation, on partial acetolysis of Fraction B, of α-D-(1→2)-linked mannobiose, mannotriose, and mannotetraose shows that the polysaccharide contains side chains of α-D-mannopyranosyl, *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl, and *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl structures. Each of these are likely attached to the main chain by (1→2) linkages. These data agree with methylation results which indicated the presence of D-mannopyranosyl units that are nonreducing ends, 2-*O*-substituted and 2,6-di-*O*-substituted, and with the ^{13}C-n.m.r. spectrum which contained C-1 signals at δ_c 103.6, 102.2, and 99.9 (Fig. 1, B) corresponding to each of these structures with the α-D configuration[32].

The D-galactosyl units form, because they were removed by partial hydrolysis, side chains attached to a central portion of the polymer, which consists of α-D-mannopyranosyl units. The presence of a large C-1 signal at δ_c 108.4 in the ^{13}C-n.m.r. spectrum is characteristic of β-D-galactofuranosyl units. Low-field values of δ_c 106.6–109.3 have been reported[30,31,35–38] for such structures in other polysaccharides. These contrast with $\delta_c \sim 101$ for C-1 signals of α-D-galactofuranosyl residues of varianose[39], δ_c 103.9–105.9 for C-1 signals of β-D-galactopyranosyl units[40–42], and δ_c 100.3 (ref. 41) and 102.1 (ref. 43) for those of α-D-galactopyranosyl residues of a D-galacto-D-mannan[44]. The detection of a 2,3,6-tri-*O*-methyl derivative of galactitol in the methylation experiments indicates 5-*O*-substitution, as in the D-galacto-D-mannan of *Penicillium charlesii*. A D-galactotetraose, isolated from the polysaccharide, gave a C-1 signal at δ_c 108.6*, corresponding to nonreducing ends and 5-*O*-substituted β-D-galactofuranosyl units[30] in the side units.

The average length of the (1→5)-linked β-D-galactofuranosyl side-chains was estimated as ~4 units by comparing the areas of the g.l.c. peaks of 2,3,5,6-tetra-*O*- and 2,3,6-tri-*O*-methylgalactitol acetates, obtained in the methylation experiment. These areas were 14 and 41% of the total peak-areas of alditol acetates, respectively.

The length of side chains is consistent with the relative sizes of various C-1 signals of β-D-galactofuranosyl units (Fig. 1, B), if one compares the main signal at δ_c 108.4, arising from nonreducing ends, and internal units which are (1→5)-linked to other β-D-galactofuranosyl units[30], with those at δ_c 109.2 and 107.4 which corre-

*Literature value, corrected by +0.6 p.p.m. to compensate for the difference in temperature (32° *vs.* 70°) in the two experiments.

spond to β-D-galactofuranosyl units attached to the α-D-mannopyranosyl core. Recent studies have shown that with such a linkage, the C-1 shift of the β-D-galactofuranosyl unit is dependent on the position of substitution of the glycosidic linkage on the α-D-mannopyranosyl unit. Methyl glycosides of β-D-galactofuranosyl-α-D-mannopyranoses had C-1′ shifts of δ_c 106.5 for (1→3) linkage, δ_c 107.7 for (1→2) linkage, and δ_c 109.7 for (1→6) linkage[31]. Thus, in the light of the methylation data, which indicate 2-*O*-, 6-*O*-, and 2,6-di-*O*-substituted D-mannopyranosyl units, the C-1 signal at δ_c 109.2 shows that the main linkage between D-galactosyl and D-mannosyl units is (1→6), and the very small signal at δ_c 107.4 may arise from a unit involved in a (1→2) linkage.

These data suggest that the Fehling-insoluble galactomannan of hyphae of *A. niger* has a chemical structure containing components **1** and **2**. On the basis of their closely related ^{13}C-n.m.r. spectra (Fig. 2), D-galacto-D-mannans of *A. terreus*, *A. flavus*, *A. fumigatus*, and *A. nidulans*, obtained by alkaline extraction and purification by Cetavlon precipitation in the presence of borate, contain similar structural features.

Such structures differ markedly from those proposed by Bardalaye and Nordin[16] for the alkali extract of the cell wall of *A. niger*: a main chain containing α-D-mannopyranosyl units having one to three successive (1→2) linkages interspersed with a single (1→6) linkage; the (1→6) linked units are substituted in the 2-positions by side chains having the sequence β-D-Gal*f*-(1→4)-(D-Gal*p*)$_{1-2}$-(1→4)-D-Gal*p*-(1→. This structure was partly based on methylation data, similar to those described herein, except that *O*-methylglucitol derivatives were detected as minor components[16], and that only 15–18% of the polysaccharide was cleaved[16] by exo-β-D-galactofuranosidase[33]. This percentage agrees with the liberation of 15% of the polysaccharide in the form of monosaccharide, on mild hydrolysis at pH 2.0 for 40 min at 100°. Methylation analysis of the resulting acid-degraded polysaccharide showed an 85% reduction in 2,3,5,6-tetra-*O*-methylgalactitol acetate corresponding to D-galactofuranosyl end-units. However, in the present investigation we found that even after 18 h at pH 2 and 100° the D-galactofuranosyl residues were not all removed, as shown by methylation analysis of the degraded polysaccharide, which gave rise to 2,3,5,6-tetra-*O*-methylgalactitol acetate (a peak of 15% of the total area).

Thus, the linkage between the β-D-galactofuranosyl unit and the D-mannan core, which is (1→6), is more stable to acid than the (1→5) linkages between the β-D-galactofuranosyl units. Thus, the evidence presented herein shows that, under our culture conditions, D-galactopyranosyl units do not exist in polysaccharides extracted from *A. niger*, except as components of 2-amino-2-deoxy-D-galacto-D-galactan that contains (1→4)-linked α-D-galactopyranosyl residues[11].

Growing fungi at different temperatures often results in the synthesis of polysaccharides having different structures. The ascomycete *Ceratocystis paradoxa* synthesizes an alkali-extractable D-mannan at 37°, whereas at 25° a D-gluco-D-mannan is formed. In this study[45], it was also found that 2-acetamido-2-deoxy-glucosyl units were present in *Ceratocystis* polysaccharides obtained at 37° but not

at 25°. Structural differences in the side chains of rhamnomannans from *Sporothrix schenckii* grown at 25° and 37° were also observed, but in this case a morphological phase-transition took place[36,46]. In our *Aspergillus* spp. some differences were observed in the proportions of monosaccharides of the alkali-extracted, partially purified polysaccharides from cells grown at 25 or 37°. However, based on ^{13}C-n.m.r. spectroscopy, the polysaccharide structures formed at both temperatures are very similar, suggesting that this phenotype is a stable characteristic of species of *Aspergillus* which usually grow well over a wide temperature-range. Common structures in the D-galacto-D-mannans from *A. niger* and the other species of *Aspergillus* may preclude the use of this antigen for the specific identification of *A. fumigatus*, the most common agent of human aspergillosis, unless the spore polysaccharides have markedly different chemical structures. Weak or strong cross-reactivities of D-galacto-D-mannans from *Aspergillus* and other fungal species have already been reported[14,15] by use of hyperimmune sera.

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