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A (1→3)-α-D-GLUCAN ISOLATED FROM THE FRUIT BODIES OF *Lentinus edodes*

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ABSTRACT

An alkali-soluble glucan, $[\alpha]_D$ +258° (M sodium hydroxide), was isolated from the fruit bodies of *Lentinus edodes*. On the basis of methylation analysis, periodate oxidation, and fragmentation analysis by partial acid-hydrolysis, this glucan was shown to have a slightly branched structure composed of (1 → 3)- and (1 → 4)-linked, α-D-glucopyranose residues in the ratio 5.3:1. The glucan was partially degraded by amylolytic enzymes. Gel filtration of the glucans degraded by alpha amylolysis or by Smith degradation suggests that (1 → 4)-α-D-glucopyranose residues are not interspersed randomly, but are present in the regions not far from the non-reducing ends.

INTRODUCTION

In previous publications[1,2], we reported the isolation and structural studies of three, water-soluble polysaccharides from the fruit bodies of *Lentinus edodes*. The present paper deals with the isolation and structure of a (1 → 3)-α-D-glucan that constitutes a major polysaccharide of the fruit bodies of *Lentinus edodes*.

RESULTS AND DISCUSSION

The residue remaining, after successive extractions of dried fruit bodies of *Lentinus edodes* with trichloroacetic acid and hot water, was then extracted with M sodium hydroxide. On neutralization of the brownish extract with acetic acid, a mixture of polysaccharides was precipitated as a uniform gel. It was purified by several repetitions of the redissolution in alkali and reprecipitation with acetic acid, until the specific rotation remained constant; $[\alpha]_D$ +197° (M sodium hydroxide). The purified, polysaccharide preparation was dehydrated by treating it with methanol and ether, and air dried (yield, 5.9% on the basis of the dry fruit bodies). On acid hydrolysis, it gave rise to D-glucose, D-mannose, and D-xylose, which were detected by paper chromatography. Fractional precipitation of this polysaccharide preparation with methanol yielded a pure glucan, $[\alpha]_D$ +258° (M sodium hydroxide), which was precipitated from an alkaline solution at a concentration of methanol of 50–56.5%

TABLE I

COMPONENT SUGARS OF THE SUBFRACTIONS OBTAINED BY FRACTIONAL PRECIPITATION WITH METHANOL

Fraction	*Weight (mg)*	$[\alpha]_D$	*Component sugars*[a]		
			Glc	*Man*	*Xyl*
Original sample	1000	197	++	+	+
P-1	258	192	+	+	+
P-2	384	196	++	+	+
P-3	228	258	++	—	—

[a] ++, Present in large proportion; +, present; —, absent.

(Table I)*. The glucan thus obtained contained no nitrogen and was not stained with iodine under the standard blue-value conditions[5]. It gave only one peak on zone electrophoresis (Fig. 1) and eluted as a single peak on gel filtration with Sepharose CL-6B (Figs. 2 and 3). These results indicate that the glucan is homogeneous and free from such other glucans as glycogen. Infrared absorption at 850 cm^{-1} but not at 890 cm^{-1}, together with high specific rotation, are characteristic of α-D-glucans. Moreover, the infrared spectrum, showing absorptions at 820 and 850 cm^{-1}, was similar to that of a (1 → 3)-α-D-glucan isolated from the cell wall of *Aspergillus niger*[6].

Treatment of the glucan with sodium metaperiodate led to consumption of 0.18 mol of periodate and release of 0.01 mol of formic acid per glucose residue. Reduction of the oxidized product with sodium borohydride, followed by mild acid-hydrolysis afforded a soluble product amounting to 16.2% of the glucan. Paper-chromatographic analysis of the soluble product showed that the major component

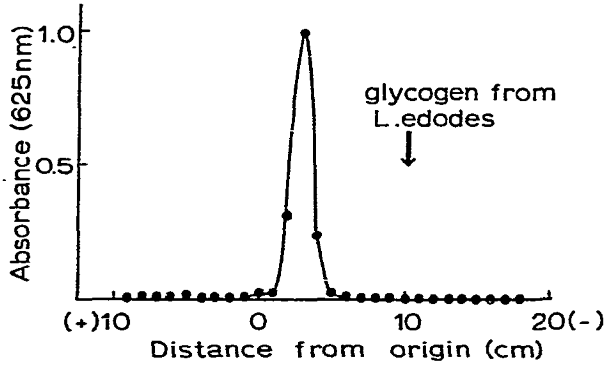

Fig. 1. Zone electrophoresis of the glucan. Electrophoresis was performed on Toyo GA-100 glass filter-paper at 90 V, 48 h, with 1:1 0.1M sodium tetraborate–M sodium hydroxide.

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*The other fractions appeared to contain a xylomannan in addition to the glucan. However, unlike *Polyporus tumulosus* xylomannan[3,4], an attempt to isolate this xylomannan as a copper complex has not been successful.

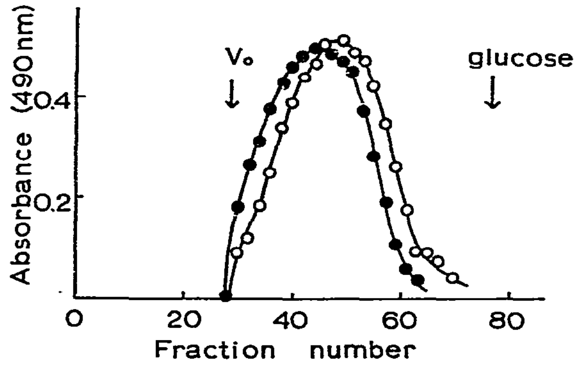

Fig. 2. Elution profiles on a column (52 × 1.5 cm) of Sepharose CL-6B. The column was eluted with 0.1M sodium hydroxide.
●—● glucan; ○—○ Smith-degraded glucan.

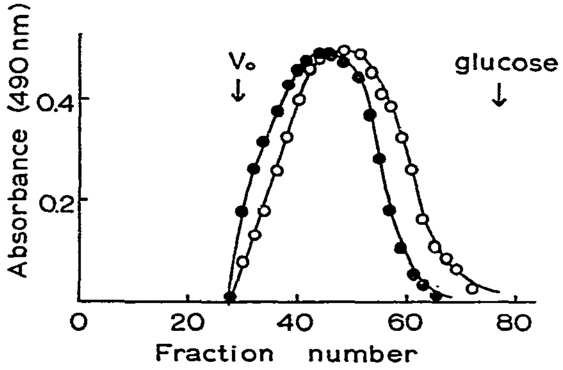

Fig. 3. Elution profiles on a column (52 × 1.5 cm) of Sepharose CL-6B. The column was eluted with 0.1M sodium hydroxide.
●—● glucan; ○—○ alpha amylase-degraded glucan.

was erythritol, together with a trace of glycerol. Non-hydrolyzed material was recovered in 83.8% yield and was resistant to further oxidation. On acid hydrolysis, the remaining core-material afforded only glucose, which was detected by paper chromatography. These results suggest that the major linkage of this glucan is (1 → 3), although the occurrence of a lesser proportion of (1 → 4)-linkages was demonstrated. The fact that no glucosylerythritol was detected in the Smith-degradation product suggests the absence of alternate (1 → 3)- and (1 → 4)-linkages. The Smith-degraded glucan was subjected to gel filtration with Sepharose CL-6B (Fig. 2). As compared with the native glucan, the elution position of the degraded glucan was slightly delayed, without any noteworthy change of polydispersity. These results indicate that breakdown of the macromolecule does not take place to a great extent during the Smith degradation, despite the apparent lowering of the average mol.wt.

The glucan was methylated by successive methylation by the procedures of Haworth (once) and Hakomori (three times), after which time the product showed

TABLE II

HYDROLYSIS PRODUCTS FROM THE METHYLATED GLUCAN

Methylated sugars	*Weight (mg)*	*Molar percent (%)*	*T*[b]
2,3,4,6-Me_4-Glc[a]	6.20	1.9	1.00
2,4,6-Me_3-Glc	285.92	78.5	1.85
2,3,6-Me_3-Glc		14.7	2.31
2,3-Me_2-Glc	14.08	4.9	

[a]2,3,4,6-Me_4-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose. [b]Retention times of the corresponding alditol acetates on an ECNSS-M column at 190°, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

TABLE III

OLIGOSACCHARIDES FROM THE PARTIAL HYDROLYZATE

Oligosaccharide		*Yield (g)*[a]	$[\alpha]_D$	*β-Acetate*		R_G[b]	*Partial hydrolysis*[c]
				$[\alpha]_D$	*m.p.*		
Nigerose	(A)	8.6	135	82	148–150	0.81	Glc, Nig
Maltose	(B)	1.3	127	61	158–159	0.74	Glc, Mal
Nigerotriose	(C)	2.0	180	106	188–189	0.64	Glc, Nig, C.
Maltotriose	(D)	0.2	154			0.54	Glc, Mal, D.
Nigerotetraose	(E)	1.6	203			0.47	Glc, Nig, C, E.
Nigeropentaose	(F)	1.6	206			0.35	Glc, Nig, C, E, F.

[a]Crude yield. [b]Relative to glucose; solvent *C*, with double development. [c]Nig = nigerose, Mal = maltose.

no i.r. absorption for free hydroxyl groups. Hydrolysis of the methylated glucan, $[\alpha]_D$ +174° (chloroform), yielded a mixture of methylated sugars that was separated by preparative paper-chromatography to give the methyl ethers listed in Table II. The methylated sugars were characterized by comparison with authentic specimens by paper chromatography or paper electrophoresis, and by g.l.c. of the alditol acetates. The inconsistent molar proportions of tetra-*O*-methylglucose and di-*O*-methylglucose might arise from the poor recovery of the former because of its relatively high volatility. The results of methylation analysis showed that the glucan has a slightly branched structure composed of (1 → 3)- and (1 → 4)-linked D-glucopyranose residues in the ratio 5.3:1.

Partial acid-hydrolysis of the glucan afforded a mixture of oligosaccharides that was fractionated on a column of charcoal–Celite. The fractionated oligosaccharides were further purified by charcoal–Celite column chromatography in the presence of borate, by gel filtration with Bio-Gel P-2, by column chromatography with Dowex-1 X-2 (borate form), and by preparative paper-chromatography. Six oligosaccharides were isolated and characterized, as listed in Table III. Isolation of maltose and maltotriose, in addition to nigero-oligosaccharides, substantiates the results of periodate oxidation and methylation analysis.

In order to obtain further information on the fine structure of the glucan, it was treated with amylolytic enzymes. On treatment with amylases, small proportions of reducing sugars were liberated from the glucan. The degree of hydrolysis with alpha amylase, calculated on the basis of reducing activity, was 4%. With beta amylase and glucoamylase, the values were 1.5 and 3.2%, respectively. Susceptibility of this glucan to *exo*-type enzymes indicates that (1 → 4)-linkages are not exclusively present in the main chain, but, in part, may constitute side chains. On gel filtration with Sepharose CL-6B, the alpha amylase-degraded glucan was eluted in almost the same pattern as that of the Smith-degraded glucan (Fig. 3). After repeated treatment of the glucan with alpha amylase, the unaffected product, $[\alpha]_D$ +256° (M sodium hydroxide), was recovered in 77.2% yield. Methylation analysis of this product showed that the degraded glucan contained only 4% of the (1 → 4)-linkage. On periodate oxidation, the degraded glucan consumed only 0.03M of periodate per glucose residue. Borohydride reduction, followed by mild acid hydrolysis, of the "polyaldehyde" yielded a water-soluble product corresponding to 2.7% of the polyalcohol. Paper-chromatographic analysis of this product showed that the main component was erythritol. Thus, the alpha amylase-degraded glucan was almost exclusively composed of (1 → 3)-linkages, although a small proportion of (1 → 4)-linkages still remained.

On the basis of the foregoing results, the alkali-soluble *Lentinus edodes* glucan was thus shown to consist of a (1 → 3)- and (1 → 4)-linked α-D-glucopyranose backbone, partially branched O-6. The (1 → 4)-linked α-D-glucose residues, present in low proportion, are not interspersed randomly, but are present in the regions not far from the non-reducing ends.

Glucans composed mostly of α-(1 → 3)-linked residues have been isolated from the fruit bodies of *Polyporus tumulosus*[3,4] and *Polyporus betulinus*[7], and from the cell walls of *Aspergillus niger*[8-10] and *Aspergillus nidulans*[11]. Bacon *et al.*[6] suggested the presence of (1 → 3)-α-D-glucan in the cell walls of certain yeasts, such as *Cryptococcus* or *Schizosaccharomyces*, by comparison of their i.r. spectra with that of an α-glucan isolated from the cell wall of *Aspergillus niger*. In a survey of the i.r. spectra, they also suggested the presence of an (1 → 3)-α-D-glucan in *Lentinus* species. However, this glucan was not investigated further.

Although these glucans contained mostly (1 → 3)-linked residues, the occurrence of a small proportion of (1 → 4)-linked residues in the glucan of *Aspergillus niger* was already noticed in the earliest paper of Johnston[8]. Horisberger *et al.*[10] have reinvestigated the structure of a (1 → 3)-α-D-glucan (pseudonigeran[12]), isolated from the cell wall of another strains of *Aspergillus niger*. Occurrence of a small proportion of (1 → 4)-linked residues (~1.4%) was demonstrated by Smith degradation of pseudonigeran. However, no definite evidence could be advanced as to whether this minor linkage was a true structural component of this glucan, or merely arose from contamination of nigeran. The latter polysaccharide is known to be a glucan composed of alternating (1 → 3)- and (1 → 4)-linked residues. Buck and Obaidah[13,14] have recently isolated an alkali-soluble glucan from the cell wall of a pathogenic fungus, *Fusicoccum amygdali*. Results from structural studies have shown that this glucan consisted of linear chains of α-(1 → 3)-linked residues, with blocks of one or two α-(1 → 4)-linked residues interspersed at intervals along the main chain. This glucan was not affected by amylolytic enzymes. However, as this glucan was extracted from the cell-wall fraction previously treated with alpha amylase, the possibility exists that the native glucan may have a structure susceptible to amylolytic enzymes. Provided that the native glucan was susceptible to amylolytic enzymes, the most serious difference between the structures of this glucan and *Lentinus edodes* glucan lies in the fact that the former is not branched at all. More recently, an alkali-soluble glucan was isolated from oak-lichen[15]. On the basis of structural studies, this glucan was shown to have a main chain composed of (1 → 3)- and (1 → 4)-linked α-D-glucopyranose residues in the ratio 6:1, with branches at O-6 of every (1 → 4)-linked residue. The ratio of (1 → 3)- and (1 → 4)-linkages of this glucan seems similar to that of our glucan. However, the fine structures of these two glucans are different. Whereas all of the (1 → 4)-linked glucose residues of oak-lichen glucan are branched at C-6, these residues are not always branched in our glucan.

From the foregoing results, the structure of *Lentinus edodes* (1 → 3)-α-D-glucan seems, therefore, to be distinct from any other related glucans. Interestingly, there seems to be some heterogeneity in the fine structures of (1 → 3)-α-D-glucans. However, the question whether this heterogeneity is related to the biological activity of different species remains unsolved.

EXPERIMENTAL

Materials. — Dried fruit bodies of *Lentinus edodes* were obtained as a commercial product purchased in a Japanese supermarket. Salivary alpha amylase was obtained from human saliva (crude, 87.8 unit/mg). Beta amylase was obtained from barley (crude, Sigma Chemical Company). Glucoamylase was obtained from *Rhizopus niveus* (pure grade, 25.7 unit/mg, Seikagaku Kogyo Co.).

General methods. — All evaporations were performed under diminished pressure at 40–45°. Specific rotations were determined at 15–20° with Nippon Bunko Model DIP-SL polarimeter. Melting points are not corrected. Infrared spectra were recorded on a Nippon Bunko IR-S infrared spectrometer. Paper chromatography was performed on Toyo No. 50 filter paper by ascending or descending methods, with the following solvent systems (v/v): (*A*) 4:1:5 1-butanol–ethanol–2% aqueous ammonia, (*B*) 5:2:7 ethyl acetate–pyridine–water, and (*C*) 6:4:3 pyridine–1-butanol–water. Preparative paper-chromatograms were conducted on Toyo No. 514 thick filter-paper. Chromatograms were sprayed with aniline hydrogen phthalate[16] for reducing sugars and alkaline silver nitrate[17] for sugar alcohols. Paper electrophoresis was carried out on Toyo No. 50 filter paper at 600 V for 3 h in borate buffer (pH 10). Gas–liquid chromatography (g.l.c.) was conducted on a Yanagimoto

Model G-80 gas chromatograph fitted with a flame-ionization detector. The glass column (225 × 0.4-cm diameter) was packed with 3% ECNSS-M on Gas Chrom Q and operated at 190° with a gas flow-rate of 20 ml of nitrogen per min. Zone electrophoresis of the polysaccharides was conducted on Toyo GA-100 glass filter-paper (5 × 60 cm) in 1:1 0.1M sodium tetraborate–M sodium hydroxide at 90 V for 48 h. The carbohydrates were detected as described in the previous paper[2].

Complete acid hydrolysis of the polysaccharides was effected by heating the sample (10 mg) in 90% formic acid (0.2 ml) for 10 min at 100°, followed by heating for additional 4 h at 100° with M sulfuric acid (1 ml). After hydrolysis with formic acid, this acid was removed by evaporation before treatment with sulfuric acid. Partial acid hydrolysis of the oligosaccharides was performed by heating the sample (2 mg) with 0.2M sulfuric acid (0.2 ml) for 1 h at 100°. Enzymic hydrolysis of oligosaccharides with glucoamylase was performed by incubating the sample in 0.05M acetate buffer (pH 5.0) for 24 h at 55°. Total sugars were estimated by the phenol–sulfuric acid method[18]. Reducing sugars were determined by the method of Somogyi–Nelson[19,20].

Isolation of α-D-glucan. — The dried fruit bodies (1 kg) were successively extracted once with 3% trichloroacetic acid (15 l) and twice with hot water (15 l), as described in the previous paper[1,2]. The residual material was suspended in M sodium hydroxide (15 l) for 2 h at room temperature under nitrogen. The alkali suspension was centrifuged, and the resulting brownish extract was neutralized with acetic acid. The precipitated polysaccharide was collected by centrifugation. The precipitate was redissolved in M sodium hydroxide, a small amount of insoluble material was removed by centrifugation, and the supernatant was neutralized with acetic acid. The same treatment was repeated five times and the precipitate was washed successively with water, methanol, and ether, and air dried; yield 59.1 g. On hydrolysis, this preparation gave rise to D-glucose, D-mannose, and D-xylose, which were detected by paper chromatography (solvent system *C*).

An α-glucan was obtained by fractional precipitation with methanol. The polysaccharide preparation (1 g) was dissolved in 0.5M sodium hydroxide (100 ml). Methanol (77 ml) was added dropwise to the solution with stirring. The precipitate (P-1) was treated with methanol, and ether (258 mg). To the supernatant, methanol (23 ml) was added as before. The insoluble fraction (P-2) was isolated (384 mg), and methanol (30 ml) was further added to the supernatant until precipitation was complete (P-3, 228 mg). On hydrolysis, P-3 gave rise only to D-glucose. The purified glucan had $[\alpha]_D$ +258° (*c* 1.0, M sodium hydroxide), contained no nitrogen, and was not stained by iodine under standard blue-value conditions[5]. The results of the fractionation are given in Table I.

Gel filtration. — A solution (0.5%) of the polysaccharide in 0.1M sodium hydroxide was applied to a column (52 × 1.5 cm) of Sepharose CL-6B. The column was equilibrated and eluted with 0.1M sodium hydroxide, and the effluent was collected in 1.2-ml fractions. The carbohydrate content of each fraction was determined by the phenol–sulfuric acid method.

Methylation analysis. — A sample of the glucan acetate (1.5 g) was methylated first by the Haworth procedure[21] and then three times by the Hakomori procedure[22]. The methylated product was extracted with chloroform and the extract was evaporated to dryness; yield 1.1 g ($[\alpha]_D$ +174°). The product showed no absorption for free hydroxyl groups in its i.r. spectrum. The methylated glucan (400 mg) was hydrolyzed with sulfuric acid according to the method of Garegg and Lindberg[23]. The neutralized hydrolyzate was deionized, and evaporated to a syrup (345 mg). The sugars were resolved by preparative paper-chromatography with solvent *A*. For g.l.c. analysis, the sugars were converted into their alditol acetates[24]. The di-*O*-methylglucose fraction migrated with the same M_G value (0.10) on paper electrophoresis as that of authentic 2,3-di-*O*-methyl-D-glucose (lit.[25] M_G 0.12). A negative reaction with the triphenyltetrazolium reagent[26] proved HO-2 to be substituted. The results and molar percentages are given in Table II.

Periodate oxidation and Smith degradation. — The glucan (100 mg) was suspended in 0.02M sodium metaperiodate (100 ml) and kept at 5° in the dark with continuous stirring. The periodate consumption was determined by the spectrophotometric method[27], and formic acid was titrated with 0.01M sodium hydroxide after reduction of the excess periodate with ethylene glycol. The glucan consumed 0.18 mol of periodate and released only a trace (0.01 mol) of formic acid per glucose residue. After completion of the scaled-up oxidation (glucan; 660 mg), the oxidized glucan was collected by centrifugation, and washed with water. The product was suspended in water (40 ml) and reduced with sodium borohydride (100 mg) for 24 h. The excess of borohydride was decomposed by addition of acetic acid (pH 6.0). The reduced glucan was collected by centrifugation and washed with water, and then hydrolyzed with 0.1M sulfuric acid for 40 h at 27°. The remaining, insoluble glucan was collected, washed successively with water, methanol, and ether, and air dried. This product was designated as Smith-degraded glucan (502.3 mg) .The supernatant solution from the mild acid hydrolyzate was neutralized with barium carbonate, and the neutral solution was evaporated. After removal of borate by evaporation with methanol, followed by treatment with Amberlite IR-120 (H^+), the solution was evaporated to a syrup (97.2 mg, 16.2% of total recovery). Paper chromatography with solvent *B* revealed the presence of erythritol (major component) and glycerol (trace).

Partial acid hydrolysis of the glucan. — The glucan (40 g) was dissolved in 90% formic acid (600 ml) and heated for 60 min at 100°. After evaporation of the formic acid, the hydrolysis was continued in 0.4M sulfuric acid (6 l) for 90 min at 100° (degree of hydrolysis, 52%). After neutralization with barium carbonate, the solution was concentrated and the resulting sugar mixture was resolved by chromatography on a charcoal–Celite column (300 g of each). The column was eluted successively with water and aqueous ethanol (5–20%). The effluent was collected in 2-l fractions, which were examined by paper chromatography (solvent *C*). Chromatographically identical fractions were combined and evaporated to dryness.

Characterization of the hydrolysis products. — Fractions 6–10, eluted with

5–10% ethanol, yielded 9.9 g of amorphous powder on evaporation. This product showed two spots (R_G 0.80 and 0.74) on a paper chromatogram (solvent *C*) and M_G 0.68 and 0.31 on a paper electrophoretogram. A portion (700 mg) of this fraction was chromatographed on a charcoal–Celite column (15 g of each). The column was successively eluted with 0.05M sodium tetraborate containing ethanol (0–1.0%). The effluent was collected in 200-ml fractions and two peaks emerged: component A at 0%, and component B at 1.0% of ethanol. The material was deionized with Amberlite IR-120 (H^+) and boric acid was removed by repeated evaporation of methanol from the product. Component A (472 mg) showed the same R_G and M_G values as nigerose and had $[\alpha]_D$ +135° (*c* 0.5, water). It yielded a β-octaacetate having $[\alpha]_D$ +82° (*c* 0.5, chloroform) and m.p. 148–150°, undepressed on admixture with an authentic specimen. Component B (69 mg) showed the same R_G and M_G values as maltose and had $[\alpha]_D$ +127° (*c* 0.5, water). It gave a β-actaacetate having $[\alpha]_D$ +61° (*c* 0.5, chloroform) and m.p. and mixed m.p. 158–159°.

Fraction no. 11, eluted with 10% ethanol, yielded 1.6 g of amorphous powder, which showed two components by paper chromatography. The major component had R_G 0.64 and the minor one had the same R_G value as that of nigerose. A portion (100 mg) of this fraction was chromatographed on a column (188 × 1.6 cm) of Bio-Gel P-2. The column was eluted with water and two peaks emerged. The major component (81 mg; C) showed a single spot (R_G 0.64) on the paper chromatogram. This component had $[\alpha]_D$ +164° (*c* 1.0, water) and yielded a crystalline β-acetate having $[\alpha]_D$ +106° (*c* 0.5, chloroform) and m.p. 188–189°. Although the $[\alpha]_D$ value of the acetate (+106°) is somewhat different from the published[9] value (+120°), other properties ($[\alpha]_D$ of the free sugar and m.p. of the acetate) of component C are in good agreement with those published for nigerotriose.

Fraction no. 12, eluted with 15% ethanol, yielded 1.1 g of amorphous powder, which showed the presence of two spots (papergram). A portion (100 mg) of this fraction was chromatographed on a column (10 × 1.2 cm) of Dowex-1 (X-2, BO_4^{3-}). The column was eluted with 0.025M potassium tetraborate and then with 0.05M acetic acid. The fraction eluted with 0.05M acetic acid (61 mg) showed the same properties as those of component C. Elution with 0.025M potassium tetraborate led to separation into two components. The major component (20 mg, D), eluted with 0.025M potassium tetraborate, showed a single spot on the paper chromatogram (R_G 0.54) and had $[\alpha]_D$ +154°. Hydrolysis of this component with glucoamylase gave only D-glucose. Paper electrophoresis of a partial hydrolyzate of it did not show the presence of nigerose. These properties are compatible with those reported for maltotriose. The minor component (5 mg), eluted slightly later, was indistinguishable from maltotriose by paper chromatography. However, when treated with glucoamylase, it was degraded only slightly to glucose, whereas authentic maltotriose was completely degraded under the same conditions.

Fractions 14–18, eluted with 15% ethanol, yielded 1.6 g of amorphous powder. On paper chromatography, only one major spot (R_G 0.47) was detected. However, treatment of this fraction with glucoamylase yielded a small proportion of nigerotriose and glucose. A portion (100 mg) of this fraction was incubated with glucoamylase. The digest was separated by paper chromatography and the zone corresponding to the spot having R_G 0.47 was cut from the sheet and eluted with water. The eluate was evaporated to give 35 mg of component E, which had $[\alpha]_D$ +203° (*c* 0.5, water) and showed a single spot on paper chromatography. Fractions 19–21, eluted with 20% ethanol, yielded 1.6 g of amorphous powder. The fractions were treated as before and the component F thus isolated had $[\alpha]_D$ +206° (*c* 0.5, water), R_G 0.35. The properties of components E and F are in fairly good agreement with values published for nigerotetraose and nigeropentaose, respectively[9]. On partial acid hydrolysis, components C, E, and F were found to yield the preceding members of the series, as listed in Table III.

Action of enzymes on the glucan. — The glucan (40 mg) was incubated with alpha amylase (0.5 mg) in 50 ml of 0.02M phosphate buffer (pH 6.8) at 37°. The glucan (40 mg) was also incubated with beta amylase (1 mg) in 0.1M acetate buffer (pH 4.7) at 35° and with glucoamylase (2 mg) in 0.04M acetate buffer (pH 5.0) at 55°. Samples were taken at intervals, and the reducing power was determined.

Properties of the alpha amylase-degraded glucan. — The alpha amylase-degraded glucan was prepared by repeated (twice) incubation of the glucan (500 mg) with alpha amylase (10 mg each time) for 24 h. The mixture was centrifuged and the insoluble material was washed with water, methanol, and ether, and air dried; yield 386 mg, $[\alpha]_D$ +256° (M sodium hydroxide). By methylation analysis of the degraded glucan, 4% of 2,3,6-tri-*O*-methyl-D-glucose was detected. On periodate oxidation, the degraded glucan consumed only 0.03M of periodate per glucose residue. Borohydride reduction of the oxidized product, followed by mild acid hydrolysis afforded a water-soluble product corresponding to 2.7% of the glucan. Paperchromatographic analysis of the soluble product showed that the major component was erythritol.

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