The Partial Acid Hydrolysis of a Highly Dextrorotatory Fragment of the Cell Wall of *Aspergillus niger*

ISOLATION OF THE α-(1→3)-LINKED DEXTRIN SERIES

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(*Received* 25 *January* 1965)

1. A highly dextrorotatory polysaccharide (α_D +232° in N-sodium hydroxide), previously isolated as a fragment of *Aspergillus niger* cell walls, was prepared from whole mycelium and subjected to partial acid hydrolysis. 2. Fractionation of the hydrolysate on a charcoal column with a linear gradient of ethanol yielded a series of oligosaccharides. The disaccharide member was shown to be nigerose (3-*O*-α-D-glucopyranosyl-α-D-glucopyranose), although a small proportion of the disaccharide peak (10%) was present as maltose despite the fact that all the nigeran had been removed from the starting material. 3. The oligosaccharides forming the main peaks from the column were shown to be members of a polymer-homologous series (nigerodextrins) by (*a*) the relationship between the logarithm of their chromatographic mobility and degree of polymerization, (*b*) obeying the Freudenberg relationship, and (*c*) partial acid hydrolysis.

In the preceding paper (Johnston, 1965) nigeran (α_D +281° in N-sodium hydroxide) and a second fraction rich in glucose (α_D +231° in N-sodium hydroxide) were shown to be present in *Aspergillus niger* cell-wall preparations. The nature of this second material, which may constitute 20–30% of the cell wall, has been investigated by partial acid hydrolysis and the results are the subject of the present paper.

METHODS

General methods. For paper chromatography solvents were: solvent *A*, ethyl acetate–pyridine–water (10:4:3, by vol.); solvent *B*, butan-1-ol–pyridine–water (6:4:3, by vol.); solvent *C*, propan-1-ol–ethyl acetate–water (6:1:3, by vol.). Paper electrophoresis was in 0·05M-sodium tetraborate adjusted to pH 10·0 with NaOH. Detecting reagents were : (1) $AgNO_3$–NaOH (Trevelyan, Procter & Harrison, 1950); (2) aniline hydrogen phthalate (Wilson, 1959); (3) aniline–diphenylamine (Schwimmer & Bevenue, 1956); (4) alkaline triphenyltetrazolium chloride (Wallenfels, 1950). In chromatography, R_{Glc} is the $R_{glucose}$ value; M_{Glc} is electrophoretic mobility relative to glucose (Foster, 1957) corrected for electroendosmosis.

Concentrations of oligosaccharides were determined after hydrolysis to glucose (1·8N-H_2SO_4 for 4hr. at 100°) and estimation of reducing power (Somogyi, 1945). In computing concentration, account was taken of the degree of polymerization and of the destruction of glucose (found to be 3%) during hydrolysis. Degrees of polymerization of oligosaccharides were determined by the method of Peat, Whelan & Roberts (1956), except that 1ml. portions of the acidified reduction mixture were analysed by using orcinol–H_2SO_4 (François, Marshall & Neuberger, 1962). It was established by paper chromatography that $NaBH_4$ did not bring about alkaline degradation of the α-(1→3)-linked oligosaccharides described in the present paper. Partial acid hydrolysis of oligosaccharides was in 0·4N-H_2SO_4 at 100° for 15min. Where purification of oligosaccharides was carried out with Whatman 3MM paper, solvent *B* was used.

β-Acetates were made by using sodium acetate and acetic anhydride (Wolfrom & Thompson, 1963). They were recrystallized at least twice from ethanol or aqueous ethanol. Melting points are not corrected.

Sugar syrups were treated with ethanol to about 70–80% concentration to remove turbid materials. Solutions were then clarified by centrifuging at up to 13000 rev./min. on an Angle 17 centrifuge (Measuring and Scientific Equipment Co. Ltd., Crawley, Sussex). Deionization was effected with De-Acidite FF (CO_3^{2-} form; 2% cross-linking) and Amberlite IR-120 (H^+ form; 8% cross-linking). Formic acid was removed from eluates with De-Acidite FF (CO_3^{2-}).

Growth of A. niger (*strain no.* 17454, *Commonwealth Mycological Institute, Kew, Surrey*). Spores washed off slopes of Czapek–Dox agar and germinated at 32° (see Johnston, 1963) were used to inoculate (1l. of inoculum) the following medium: $NaNO_3$, 150g.; KCl, 37·5g.; magnesium glycerophosphate, 37·5g.; $FeSO_4$, 0·75g.; K_2SO_4, 26g.; sucrose, 2250g.; distilled water to 75l. Growth was continued for 67hr. at about 28–30° in a glass-lined fermentation vessel with a paddle stirring at 120 turns/min. and a sterile air flow of approx. 40l./min. The yield of washed mycelium was 11kg. wet wt. This large-scale growth was kindly carried out by Mr R. P. Legge and Dr M. D. Lilly of the Department of Chemical Engineering, University College, London.

Isolation of fraction IV R (*Johnston*, 1965). The material was isolated from the whole mycelium as follows. About 6·5kg. wet wt. of mycelium was distributed in 10l. of distilled

water and autoclaved for 5–10 min. at 10 lb./in.^{2}. The liquid was filtered hot through muslin, and, when cool, the flocculent nigeran precipitate (α_D +281° in N-NaOH) was collected. After five such extractions with water the mycelium was then extracted with N-NaOH (4 l.) with stirring and under a stream of N_2. After this, the mycelium was separated by centrifuging (4 × 1 l. pots on a Measuring and Scientific Equipment Co. Ltd. Major centrifuge; 1000*g* for 20 min.) and re-extracted with N-NaOH (3 l.). The combined supernatants, after filtration through a no. 1 sinter to remove any particles of mycelium, were neutralized with acetic acid. The resulting precipitate was washed free of salts by resuspending in water and centrifuging as above (about four washes were required). The precipitated material was suspended with stirring in 40 mM-$NaHCO_3$, pH 8·1 (4 l.), and digested for 18 hr. at 37° with 100 mg. of crystalline trypsin (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) in the presence of a small amount of chloroform. It was then washed by centrifuging three times with water (4 × 1 l.), dissolved in N-NaOH ([α]$_D$ approx. +240°), reprecipitated with acetic acid and finally washed free of salt. It still contained nigeran and therefore was autoclaved a further three times with water (9 l. each time) until the supernatant gave no reaction for carbohydrate (phenol–H_2SO_4 test; Dubois, Gilles, Hamilton, Rebers & Smith, 1956). The residue was freeze-dried, refluxed with boiling methanol (1·5 l. for 4 hr.), filtered on a no. 1 sinter, before finally washing with methanol and ether and sucking dry. The yield was 26·4 g. (fraction IVR; Johnston, 1965). Analysis showed: N, less than 0·02%; ash, nil; after subtraction of moisture content, the material was shown, on complete hydrolysis, to consist of 100% of carbohydrate; the mannose: galactose: glucose proportions (Wilson, 1959) were approx. 1:4:95. It had $[\alpha]_D^{27}$ +232° (*c* 0·122 in N-NaOH) compared with +231° from a cell-wall preparation. In all, from approx. 8 kg. wet wt. of mycelium, 38·4 g. of fraction IVR and 11 g. of nigeran were obtained.

Partial acid hydrolysis of fraction IVR. The fraction (10 g., moisture content 12%) was heated on a boiling-water bath in 98% formic acid (150 ml.) for exactly 10 min. and then boiling 0·4 N-H_2SO_4 (1500 ml.) was added. Heating was continued for 50 min. and then stopped. A 1 ml. portion of the hydrolysate was used for measurement of reducing power and a further 2 ml. for the determination of sugar content (as glucose) on complete hydrolysis (Somogyi, 1945). The apparent conversion into glucose was 48%. A further quantity of fraction IVR (10·6 g.) was treated similarly except that the heating after the addition of 0·4 N-H_2SO_4 was extended to 60 min., when the apparent conversion was 60%. Each hydrolysate was cooled rapidly and neutralized with NaOH, and the pooled hydrolysates were concentrated to about 1500 ml. The solution was centrifuged to remove a dark-brown gum (3% of starting material) and then loaded on to a charcoal (grade no. 130; 80–200 mesh; Sutcliffe and Speakman Ltd., Leigh, Lancs.) column (5·7 cm. × 72 cm.). The Celite expander was omitted from the charcoal column (Crook & Stone, 1957). The solution loaded contained 18 g. of carbohydrate as anhydroglucose.

Fractionation of hydrolysate. The column was washed with 0·01 N-formic acid (about 20 l.) and the monosaccharide fraction (8·0 g.) emerged after 4–5 l. It was shown by paper chromatography to contain mainly glucose, a small amount of galactose and a trace of mannose. The column was finally equilibrated by washing with 0·1 N-formic acid (5 l.; no monosaccharides emerged during this treatment) followed by 0·01 N-formic acid until the pH of the effluent was 2·9. A linear gradient of ethanol in 0·01 N-formic acid was then run on to the column (Taylor & Whelan, 1962), by using in the mixing chamber 20 l. of 0·01 N-formic acid and in the reservoir 20 l. of 0·1 N-formic acid in aq. 30% (v/v) ethanol. Fractions of volume 50 ml. were collected and the phenol–H_2SO_4 reaction was done, on 0·1–1·0 ml. portions, on every third or fourth tube. At about tube 800, the gradient was extended at the same ethanol increment (0·75%/l.) to allow 900 tubes to be collected. Eleven peaks were collected and the yields obtained were as follows: peak A, tubes 156–202, 0·116 g.; peak B, tubes 218–306, 3·225 g.; peak C, tubes 348–372, 0·076 g.; peak D, tubes 400–444, 1·973 g.; peak D_x, tubes 445–456, 0·116 g.; peak E, tubes 457–478, 0·139 g.; peak F, 486–522, 0·158 g.; peak G_1, tubes 528–548, 0·082 g.; peak G, tubes 550–600, 1·250 g.; peak I, tubes 652–688, 0·567 g.; peak L, tubes 860–880, 0·239 g.; Peaks B, D, G, I, and L accounted for 91% of the oligosaccharides recovered and were the only ones examined in the present work. The remaining peaks, obtained in yields of less than 1% of the material loaded, contained in several cases three or four components (in solvent *A*) and were not further studied. The overall recovery from the column was 15·8 g. (includes water of hydrolysis), representing 88% on the basis of material loaded.

EXPERIMENTAL AND RESULTS

Examination of peak B. Paper chromatography with solvent *A* showed the presence of two spots running in the positions of maltose (typical blue colour with reagent 3) and nigerose, the latter in greatest quantity. The presence of maltose was confirmed by paper electrophoresis. A portion (840 mg.) of peak B material was chromatographed on a charcoal column (2·3 cm. × 56 cm.) with a linear gradient of ethanol (increment 1·5%/l.) in 30 mM-sodium tetraborate (Bose, Foster, Salim, Stacey & Webber, 1961). Fractions (20 ml.) were collected into tubes holding 0·3 ml. of 25% (v/v) acetic acid. Three peaks emerged: peak 1 at 1·3–3·0%, peak 2 at 3·9–4·4%, and peak 3 at 4·8–6·3% (v/v) ethanol. The material of the pooled peaks, pH 6·5, was deionized with ion-exchange resins and boric acid was removed by repeated evaporation with methanol. The material of the peaks was finally loaded on to small charcoal columns (2·3 cm. × 16 cm.), washed with water to remove traces of glucose and eluted with 10% (v/v) ethanol. The sugars were evaporated to a syrup or freeze-dried. Yields (weight and percentage of total recovery) were: peak 1, 630 mg., 87%; peak 2, 19 mg., 2·5%; peak 3, 76 mg., 10·5%; the recovery was 86%. In a separate experiment 610 mg. (90%) of nigerose and 58 mg. (9%) of maltose were obtained from 780 mg. of peak B material. Peaks 1 and 3 were shown to be composed of pure nigerose and maltose respectively. Peak 2 material had three components (R_{Glc} values 0·55, 0·63 and 0·77 in solvent *B*) all reacting with

reagent (4) [absence of (1→2)-linkages]. Complete hydrolysis of peak 2 material showed glucose, galactose and a trace of mannose; it probably contained the disaccharides due to the minor hexose components of fraction IVR. Owing to the small amounts available and its complexity, it was not further studied.

Nigerose had $[\alpha]_D^{23}+133°$ (*c* 0·528 in water) by weight and $[\alpha]_D^{23}+137°$ (*c* 0·508 in water) by estimation. It yielded a β-octa-acetate having $[\alpha]_D^{23}+83°$ (*c* 0·485 in chloroform) and m.p. 150–152° not depressed on admixture with authentic material (kindly supplied by Dr K. Matsuda, Tohoku University, Sendai, Japan). Maltose had $[\alpha]_D^{18}+129°$ (*c* 0·58 in water; concentration calculated as monohydrate after hydrolysis to glucose); it gave a β-octa-acetate having $[\alpha]_D^{23}+63°$ (*c* 0·72 in chloroform) and m.p. and mixed m.p. 158–159°.

Examination of peak D. In solvent *B*, the major spot (R_{Glc} 0·59) was contaminated by a trace of peak D_x material (R_{Glc} 0·51). The impurity was removed by two successive runs of peak D material (400mg.) on Whatman 3MM paper. The yield of pure peak D substance was 240mg. (recovery, 60%). Peak D substance had a degree of polymerization of 3. Partial acid hydrolysis showed (solvent *A*) only glucose and nigerose (R_{Glc} 0·73) as well as unchanged peak D substance (R_{Glc} 0·50). Paper electrophoresis showed the absence of maltose from the hydrolysate, and the M_{Glc} of peak D substance, 0·64, suggested a (1→3)- or (1→6)-linkage at the reducing end (Foster, 1957). Peak D substance (20mg.) was reduced with sodium borohydride (20mg.) in water (20ml.) for 18hr. Boric acid and Na^+ were then removed; partial acid hydrolysis of the product gave only glucose and nigerose (solvents *A* and *B*).

Further evidence for the (1→3)-linkage at the reducing unit of peak D substance was obtained by using dilute sodium metaperiodate (Clancy & Whelan, 1959) under the conditions outlined by Abdullah, Goldstein & Whelan (1962). The results for one experiment (Fig. 1) show that the initial consumption was approx. 2·7moles of metaperiodate/mole of sugar alcohol; the maximum release of formaldehyde was 1·9moles/mole.

The slowly continuing consumption of metaperiodate is probably due to the oxidation of the glycosidic part of the alcohol. The occurrence of a plateau is unlikely since the over-oxidation of the substituted malondialdehyde, arising from the hexitol moiety, would be expected to follow normal oxidation (see the oxidation of maltitol by Wolfrom, Thompson, O'Neill & Galkowski, 1952).

In a separate experiment, 22·6 μmoles of peak D sugar alcohol were oxidized until metaperiodate was 5·1moles/mole of sugar alcohol. The mixture was then boiled with N-sulphuric acid for 2·5hr. Iodine was removed (with carbon tetrachloride),

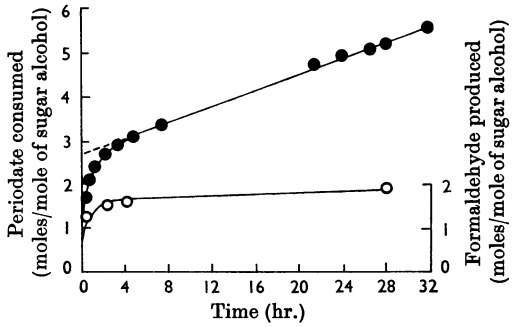

Fig. 1. Oxidation of the sugar alcohol corresponding to peak D (trisaccharide) substance with 0·4mM-sodium metaperiodate. Peak D substance (8mg.) was dissolved in water (2ml.) together with $NaBH_4$ (27mg.) and left for 24hr. Excess of borohydride was then destroyed (with H_2SO_4) and the solution diluted to about 200ml., when 5ml. of 20mM-sodium metaperiodate was added and the volume made up to 250ml. At intervals metaperiodate consumption (●) was measured on 5–10ml. portions by using 10mN-$Na_2S_2O_3$, and formaldehyde production (○) on 10–20ml. portions by using chromotropic acid (Frisell, Meech & Mackenzie, 1954).

after which deionization was effected and boric acid removed with methanol. The resulting solution, corresponding to 18·1 μmoles of the original alcohol, was analysed for glucose (glucose oxidase, EC 1.1.3.4; Huggett & Nixon, 1957) and found to contain 6 μmoles.

Peak D substance had $[\alpha]_D^{24}+184°$ (*c* 0·3 in water) and yielded a crystalline β-acetate from aqueous ethanol (Found: C, 49·9%; H, 5·6%. $C_{40}H_{54}O_{27}$ requires C, 49·7%; H, 5·6%). The crystals were extremely fine; however, they appeared crystalline under a polarizing microscope. They had $[\alpha]_D^{23}+120°$ (*c* 0·5 in chloroform) and m.p. 187–188°. The β-benzoate of peak D substance was prepared according to the method of Turvey & Whelan (1957). It failed to crystallize from several solvents during a period of months.

Examination of peak G. On paper electrophoresis a major spot (M_{Glc} 0·62 and two minor ones (M_{Glc} 0·55 and 0·43) were seen. Peak G material (266mg.) was therefore run on a charcoal column with a linear gradient of ethanol (increment 0·7%/l.) in sodium tetraborate, essentially as for the isolation of nigerose. The major component emerged between 6·3 and 9·1% and the minor one between 9·2 and 10·1% (v/v) ethanol. The latter component was not further studied. The major component was deionized on a column of charcoal (see Bose *et al.* 1961) and then eluted with 30% (v/v) ethanol. Other trace impurities of low R_F were removed by fractionation on Whatman 3MM paper. The yield of pure peak G substance was 143mg. (recovery, 54%).

On complete hydrolysis, peak G substance contained only glucose (solvent *B*). A partial acid hydrolysate showed glucose, nigerose, peak D substance and unchanged peak G substance. Peak G substance was found to have a degree of polymerization of 4.

On borohydride reduction and metaperiodate oxidation, with similar quantities as for peak D material, the tetrasaccharide alcohol gave rise to 1·74moles of formaldehyde/mole after 3·75hr. (accompanied by the consumption of 3·34moles of metaperiodate/mole). After 20hr. the formaldehyde output had risen to 2·02moles/mole of tetrasaccharide alcohol.

Despite repeated attempts, the β-acetate of peak G substance failed to crystallize from several systems, in some cases even after several months. Peak G substance had $[\alpha]_D^{26} + 205°$ (*c* 0·24 in water).

Examination of peaks I and L. By chromatography of peak I material (152mg.) on Whatman 3MM paper, traces of slower-running substances were removed from the main component (R_{Glc} 0·33). The yield of pure peak I material was 100mg. (recovery, 67%). Peak L material yielded two components, R_{Glc} 0·24 and 0·16, the slower running of which was present in smaller amount. By chromatography on paper (118mg. of peak L material) they were separated to give 62mg. of L_1 substance and 21mg. of L_2 substance (recovery, 70%).

Peak I substance contained only glucose. It had a degree of polymerization of 5. A partial acid hydrolysate showed glucose, nigerose, peak D substance, peak G substance and unchanged peak I substance. On borohydride reduction and metaperiodate oxidation (as for peak D substance), peak I alcohol showed a rapid initial production of 1·75moles of formaldehyde/mole of pentasaccharide in 3·75hr. (metaperiodate consumption was 2·92moles/mole in 3·25hr.) rising to 2·09moles/mole in 20hr. Peak I substance showed $[\alpha]_D^{23} + 223°$ (*c* 0·16 in water).

On partial acid hydrolysis L_1 substance and L_2 substance were also found to yield the preceding members of the series, L_1 substance appearing to be the hexasaccharide (it was also shown to have a degree of polymerization of 6) and L_2 substance the heptasaccharide. However, L_1 substance contained a trace and L_2 substance a slightly greater quantity of mannose. In addition to the main series of nigerodextrins produced from L_2 substance, smaller amounts of other oligosaccharides occurred. The heptasaccharide was therefore less homogeneous than other members of the series. The L_1 substance had $[\alpha]_D^{24} + 233°$ (*c* 0·18 in water) and L_2 substance had $[\alpha]_D^{24} + 235°$ (*c* 0·1 in water).

Purity of isolated oligosaccharides. The sugar contents of the nigerodextrins, determined by hydrolysis to glucose, were: di-, 97%; tri-, 93%; tetra-, 89%; penta-, 96%; hexa-, 95%; heptasaccharide, 90%. The balance was presumably inorganic material. The properties of the oligosaccharides isolated from fraction IVR are shown in Table 1.

DISCUSSION

The second polysaccharide, fraction IVR, of high dextrorotation ($[\alpha]_D + 232°$ in N-sodium hydroxide) found in the cell wall of *A. niger* has been freed as far as possible from nigeran ($[\alpha]_D + 281°$ in N-sodium

Table 1. *Properties of products of partial acid hydrolysis isolated from fraction IVR of* A. niger *cell wall*

Authentic maltose was the monohydrate; maltose in peak B was calculated, for purposes of optical rotation, as the monohydrate after hydrolysis to glucose. Degrees of polymerization were determined as described in the 'General methods' section, except for the heptaose where it was based on partial hydrolysis. F, Material from cell-wall fraction; A, authentic material. Solvent *B*, butan-1-ol–pyridine–water (6:4:3, by vol.); solvent *C*, propan-1-ol–ethyl acetate–water (6:1:3, by vol.). Values are for Whatman no. 1 paper for R_{Glc}; for M_{Glc} Whatman 3MM paper was used.

Peak	Degree of polymerization	$[\alpha]_D$ in water	β-Acetate $[\alpha]_D$ in $CHCl_3$	β-Acetate m.p.	R_{Glc} Solvent *B*	R_{Glc} Solvent *C*	M_{Glc}
B (nigerose) F	2·09	+137°	+83°	150–152°	0·76	0·82	0·70
A		—	+84	150–151	—	—	—
B (maltose) F		+129	+63	158–159	—	—	—
A		+130	+64	158			
D (nigerotriose) F	3·03	+184	+120	187–188	0·59	0·67	0·64
G (nigerotetraose) F	4·02	+205			0·44	0·51	0·62
I (nigeropentaose) F	5·03	+223			0·33	0·42	0·60
L_1 (nigerohexaose) F	6·0	+233			0·24	0·31	0·55
L_2 (nigeroheptaose) F	7	+235			0·17	0·24	0·55

hydroxide). However, since on partial acid hydrolysis the disaccharide fraction (peak B) of fraction IVR contains 9–10% of maltose together with 87–90% of nigerose, it is clear that maltosyl units still exist in fraction IVR. The relationship of these to the rest of the α-(1→3)-linked glucose residues has not been explored, and the possibility exists that they may represent a residual insoluble fragment of nigeran. Fraction IVR is obviously a polymer utilizing very largely the α-(1→3)-linkage. In view of the presence of a high proportion of α-(1→3)-linkages in fraction IVR and nigeran, the constancy of their rotations in sodium hydroxide for at least 5 days must indicate that alkaline degradation is occurring very slowly or not at all. By comparison with studies on laminarin (Corbett & Kenner, 1955), which is composed very largely of β-(1→3)-linked glucose units, sodium hydroxide, although a poorer catalyst than calcium hydroxide (Kenner & Richards, 1954), might be expected to bring about some saccharinic acid formation or other alkaline fragmentation from fraction IVR and nigeran (Whistler & BeMiller, 1958), producing a fall in rotation from the high values of +231° and +281° (see, e.g., rotations of saccharinic acids given by Sowden, 1957). Thus the terminal reducing units of fraction IVR and nigeran must be (1→2)-linked [or possibly (1→6)-linked] (Whistler & BeMiller, 1958), or be masked by a polyol, as in some laminarin molecules.

The evidence suggests that the trisaccharide peak D substance is nigerotriose [*O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→3)-α-D-glucopyranose]. The initial rapid metaperiodate consumption of approx. 3 moles/mole, together with the ultimate release of 2 moles of formaldehyde/mole of trisaccharide alcohol, is that expected for selective oxidation of a 3-*O*- or 4-*O*-substituted hexitol (Hough, Woods & Perry, 1957; Clancy & Whelan, 1959; Abdullah *et al.* 1962). The nigeran trisaccharides, *O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranose and *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→3)-α-D-glucopyranose (Barker, Bourne, O'Mant & Stacey, 1957), on reduction would yield similar values, but since it was carefully established that maltose was absent from the partial hydrolysate of peak D substance, these trisaccharides can be excluded. The production of 2 moles of formaldehyde/mole from the tetra- and penta-saccharide alcohols is also consistent with a 3-*O*-substituted reducing unit; their M_{Glc} values (0·60–0·62) also suggest this (Foster, 1957).

Partial acid hydrolysis of peak D (trisaccharide) alcohol produced only glucose and nigerose, indicating the non-reducing unit to be 3-*O*-linked. On normal oxidation, nigerotri-itol would consume 5 moles of periodate/mole, leaving the central unit unattacked. It was found that, after the consumption of 5·1 moles of metaperiodate/mole, 18·1 μmoles of peak D alcohol yielded 6 μmoles of glucose (theoretical, from 18·1 μmoles of nigerotri-itol, being 6·4 μmoles of glucose). This is consistent with, but does not prove conclusively, the 3-*O*-substitution of the central unit.

Nigerose, peak D substance, peak G substance, peak I substance, L_1 substance and L_2 substance, on the basis of their products of partial acid hydrolysis and determination of their degrees of polymerization, appear to be the di-, tri-, tetra-, penta-, hexa- and hepta-saccharide members of a polymer-

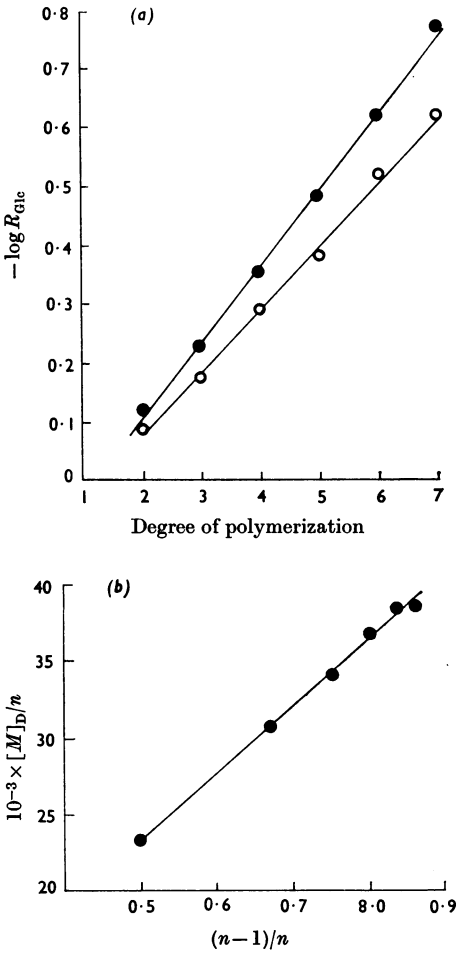

Fig. 2. (*a*) Relationship between $-\log R_{Glc}$ values and degree of polymerization for the nigerodextrins. ●, Solvent *B*, butan-1-ol–pyridine–water (6:4:3, by vol.); ○, solvent *C*, propan-1-ol–ethyl acetate–water (6:1:3, by vol.). (*b*) Application of the Freudenberg relationship to the nigerodextrins. $[M]_D$, Molecular rotation ($[\alpha]_D \times$ mol.wt.); *n*, no. of hexose units/molecule.

homologous series, in fact, the nigerodextrins, which have not been previously described, although nigerotriose has been detected in isolichenin (Peat, Whelan, Turvey, & Morgan, 1961). This view is substantiated by the linear relationship between $-\log R_{Glc}$ and degree of polymerization (Fig. 2*a*). Chromatographic mobility was plotted as $-\log R_{Glc}$ (Stone, 1954), since, for the maltodextrins in phenol–water, $\log(1/R_F - 1)$, $\log 1/R_F$ and $-\log R_{Glc}$ all show linearity with the values given in the original paper (Whelan, Bailey & Roberts, 1953). In any case it is obvious that, for a given oligosaccharide, $-\log R_{Glc}$ is related to $\log 1/R_F$. The fact that glucose does not lie on the line is of no consequence, since setting the R_F of glucose equal to 1·0 creates an arbitrary front that is constant throughout. The use of R_{Glc} also obviates the need for repeated ascending runs to obtain measurable R_F values (French & Wild, 1953) for this type of plot.

The series also obeys the Freudenberg relationship (Freudenberg, Friedrich & Bumann, 1932; Freudenberg & Blomqvist, 1935) (Fig. 2*b*). The highest members of the series have rotations just in excess of that of fraction IVR itself. However, the latter is done in N-sodium hydroxide, and, further, presence of α-(1→4)-linkages and of galactose and mannose residues must influence the rotation of the intact polysaccharide.

Since crystallization of the tetrasaccharide β-acetate was not achieved, no attempt was made to prepare the β-acetates of higher saccharides. Further, the β-benzoyl derivatives appeared to offer little hope since that of peak D substance also failed to crystallize. For the α-(1→6)-linked series (isomaltodextrins; Turvey & Whelan, 1957) no β-acetates above isomaltose are known, although the β-benzoyl derivatives of the tri- and tetrasaccharide were obtained as crystals.

Other fungal cell-wall polysaccharides on which structural information is available include yeast glucan (Bell & Northcote, 1950; Peat, Whelan & Edwards, 1958), yeast mannan (Peat, Turvey & Doyle, 1961; Peat, Whelan & Edwards, 1961), the glucan and mannan of *Candida albicans* cell wall (Bishop, Blank & Gardner, 1960) and chitin (Foster & Webber, 1960).

My thanks are due to Mr David Thomas for technical assistance and to Dr A. P. Mathias for criticizing the manuscripts of this and the preceding paper, as well as to Professor W. J. Whelan for information on running the carbon columns.

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