478. *Studies of* Aspergillus niger. *Part VI.* The Separation and Structures of Oligosaccharides from Nigeran.*

By S. A. Barker, E. J. Bourne, D. M. O'Mant, and M. Stacey.

The isolation and characterisation of nigerose (3-*O*-α-D-glucopyranosyl-D-glucose) and the two isomeric trisaccharides [*O*-α-D-glucopyranosyl-(1 → 3)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucose and *O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose] obtained from nigeran are described.

It has been shown [1,2] that the intracellular polysaccharide, nigeran, produced by *Aspergillus niger* "152," is an essentially unbranched polyglucosan in which most, if not all, of the residues are attached alternately by α-1 : 4- and α-1 : 3-linkages. We now present additional evidence for the fine structure of nigeran obtained by the separation and structural investigation of the isomeric trisaccharides derived by acidic hydrolysis. A structural investigation of the methyl furanosides of nigerose (3-*O*-α-D-glucopyranosyl-D-glucose) is also reported.

After the isolation of fractions containing mixed disaccharides and mixed trisaccharides,[1,2] the initial problem was to devise a method of separation of the isomers. Attempts to separate maltose and nigerose on Amberlite IRA-400 with a borate buffer, as used by Khym and Zill [3] for other sugar isomers, was unsuccessful owing to the alkali-lability of nigerose (cf. Corbett, Kenner, and Richards [4]). It has been shown [1,2] that the trisaccharide fraction contained two trisaccharides, one of which [T_2, presumed to be *O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose] moved at about twice the speed of the other [T_1, presumed to be *O*-α-D-glucopyranosyl-(1 → 3)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucose] on paper ionophoresis [5] in borate buffer (pH 10). Separation of bands of the trisaccharides was effected on paper in this way but elution of the strips with water and subsequent removal of the sodium and borate ions yielded degradation products, particularly in the case of T_2. Since it was shown that the procedure for removing sodium borate (cation-exchange resin and removal of boric acid by codistillation with methanol) did not cause this degradation, it was concluded that the trisaccharides were stable in borate buffer of pH 10·0 while on the paper but not in solution. Undegraded trisaccharides were however obtained by extracting the ionophoresis paper with the calculated quantity of dilute hydrochloric acid so that the resultant pH was *ca.* 5.

To obtain the larger amounts required for structural investigation a method was devised [6] which depended on the fact that one of each pair of isomers was readily converted into its methyl furanosides by treatment with methanolic hydrogen chloride at room temperature and that these furanosides were absorbed more strongly on a charcoal column than was the unchanged sugar Thus a mixture of maltose and nigerose gave chromatographically pure maltose and two fractions [(I), eluted with 10% ethanol; and (II), eluted with 30% ethanol] containing the furanosides of nigerose. These fractions had the same R_F values on a paper chromatogram and the same methoxyl contents and were non-reducing to the Shaffer–Hartmann reagent.[7] Both gave nigerose when hydrolysed with 0·01N-hydrochloric acid at 45°. When either fraction was oxidised with sodium periodate the results were in good agreement with the theoretical values for a methyl furanoside of

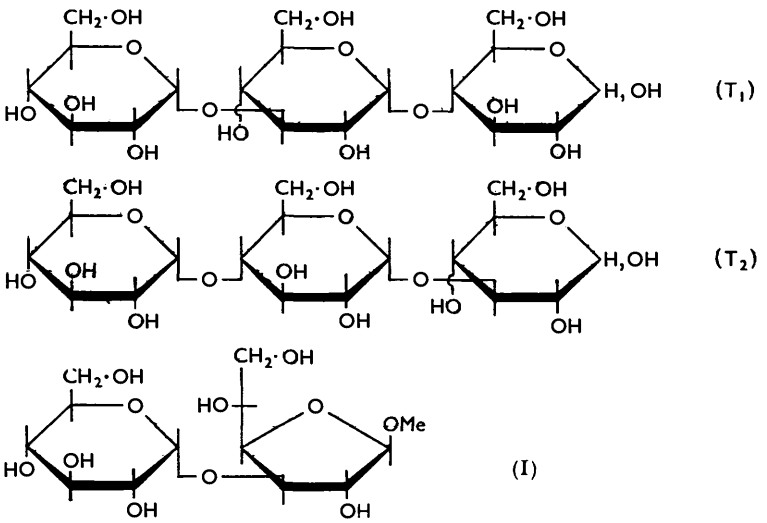
* Part V, *J.*, 1957, 2064.

[1] Barker, Bourne, and Stacey, *J.*, 1953, 3084.
[2] *Idem, Chem. and Ind.*, 1952, 756.
[3] Khym and Zill, *J. Amer. Chem. Soc.*, 1952, **74**, 2090; 1953, **75**, 1339.
[4] Corbett, Kenner, and Richards, *Chem. and Ind.*, 1953, 154.
[5] Foster, *J.*, 1953, 982.
[6] Barker, Bourne, and O'Mant, *Chem. and Ind.*, 1955, 425.
[7] Shaffer and Hartmann, *J. Biol. Chem.*, 1921, **45**, 365.

3-*O*-α-D-glucopyranosyl-D-glucose (3 mols. uptake, 1 mol. of formic acid, 1 mol. of formaldehyde). When the product of periodate oxidation of fraction (I) was reduced with sodium borohydride, hydrolysed with acid, de-ionised, and fractionated on a cellulose column, D-xylose (characterised as its crystalline β-tetra-acetate) was obtained.

Since xylose can only be formed from glucose directly by the removal of $C_{(6)}$, positions 5 and 6 must have been unblocked during the periodate oxidation, and hence the glucoside residue must have been in the furanose form and have been blocked at either position 2 or position 3. The presence of a pair of free hydroxyl groups on $C_{(5)}$ and $C_{(6)}$ having free rotation was also confirmed by the rapidity with which these were oxidised by lead tetra-acetate compared with the remaining pairs of *trans*-hydroxyl groups (cf. Hockett and McClenahan [8]).

Additional evidence for the structure of the materials (I) and (II) was obtained by methylation, hydrolysis, and fractionation of the products on a silica-gel column.[9] A chromatogram of each of the two fractions obtained showed that one was 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and that the other was a tri-*O*-methyl-D-glucose with an R_F value different from that of 2 : 3 : 5-, 2 : 3 : 6-, or 2 : 3 : 4-tri-*O*-methyl-D-glucose.



The tri-*O*-methyl-D-glucose was shown to have $C_{(4)}$ unblocked because of its ability to undergo methyl-furanoside formation in methanolic hydrogen chloride at room temperature. When the tri-*O*-methyl-D-glucose was reduced to the corresponding sorbitol derivative, the product consumed practically 1 mol. of periodate but produced no formaldehyde and a negligible amount of formic acid. The only two tri-*O*-methylsorbitol derivatives which would be expected to behave in this way and have $C_{(1)}$ and $C_{(4)}$ free were the 2 : 3 : 6- and the 2 : 5 : 6-derivative. Since however the tri-*O*-methyl sugar was distinguishable from the 2 : 3 : 6-derivative chromatographically, it must have been 2 : 5 : 6-tri-*O*-methyl-D-glucose. Ample evidence is therefore available to show that fractions (I) and (II) are predominantly the β- ($[\alpha]_D$ + 40°) and the α-methyl furanoside ($[\alpha]_D$ +128°), respectively, of 3-*O*-α-D-glucopyranosyl-D-glucose.

Separation of the trisaccharide isomers, T_1 and T_2, effected by preferential furanoside formation and fractionation on a charcoal column, gave chromatographically pure T_1 together with fractions containing the methyl furanosides of T_2 contaminated with varying amounts (7·3—28%) of T_1. The furanosides were easily freed from the T_1 by fractionation

[8] Hockett and McClenahan, *J. Amer. Chem. Soc.*, 1939, **61**, 1667.
[9] Bell, *J.*, 1944, 473.

on a cellulose column, which appears to be more efficient than a charcoal column for separating a free sugar and the furanosides of an isomeric sugar, although its capacity is more limited.

Trisaccharide T_1 was reducing and contained maltose and nigerose moieties since these disaccharides were isolated after partial acidic hydrolysis and characterised as their β-octa-acetate and osazone respectively. The sequence of linkages was determined by reduction of T_1 to the sugar alcohol and partial acidic hydrolysis of the product. Paper-chromatographic analysis showed that the only reducing disaccharide present was nigerose. This proved that T_1 was mainly *O*-α-D-glucopyranosyl-(1 → 3)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucose. In close agreement with theoretical predictions (5 mols. of periodate consumed; 2 mols. of formic acid and 2 mols. of formaldehyde produced) the alcohol of T_1 consumed 4·9 mols. of periodate and produced 2·2 mols. of formic acid and 1·9 mols. of formaldehyde. (It will also be recalled that no di-*O*-methyl glucose was detected [1] in the hydrolysate of methylated nigeran.) This evidence excludes the presence of significant quantities of a branched trisaccharide in the T_1 fraction.

The methyl furanosides of T_2 on hydrolysis with 0·01N-hydrochloric acid at 50° gave the reducing trisaccharide T_2 and on further hydrolysis (0·3N-sulphuric acid at 85—90°) produced the disaccharides maltose (characterised as its β-octa-acetate) and nigerose (characterised as its osazone), together with glucose. On oxidation with sodium periodate the results were in good agreement with the theoretical values for a methyl furanoside of *O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose (4 mols. of periodate consumed, 1 mol. each of formaldehyde and formic acid produced). Again 1 mol. of lead tetra-acetate was consumed more rapidly by the pair of hydroxyls on $C_{(5)}$ and $C_{(6)}$ of the furanoside residue than by other pairs of hydroxyls which are all *trans*. Reduction of T_2, effected under neutral conditions because of its alkali lability, and partial hydrolysis of the alcohol gave maltose and glucose as the only reducing saccharides. This confirmed the presence of the α-1 : 4-linkage at the non-reducing end. When the sugar alcohol of T_2 was oxidised with sodium periodate it consumed 6·1 mols. of periodate and produced 2·2 mols. of formic acid and 1·9 mols. of formaldehyde, in good agreement with the theoretical values of 6, 2, and 2 mols., respectively, for *O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-gluco-pyranosyl-(1 → 3)-D-glucitol.

It is evident that the structures assigned to trisaccharides T_1 and T_2 are in complete agreement with those expected from a polysaccharide in which the glucose residues are alternately linked α-1 : 3 and α-1 : 4.

Experimental

Partial Acidic Hydrolysis of Nigeran.—The polysaccharide (97 g.) was hydrolysed in a solution containing water (3·5 l.) and concentrated sulphuric acid (100 c.c.) at 85° for 3·1 hr. The reducing power of the hydrolysate then corresponded to a 47% conversion into glucose. The solution was neutralised with barium carbonate, clarified on a centrifuge, and evaporated *in vacuo* to *ca.* 200 c.c. The saccharide mixture was then fractionated on a charcoal column (*l*, 71 cm.; *d*, 5·5 cm.) by Whistler and Durso's method [10] using increasing concentrations of aqueous ethanol. Elution with water (6·7 l.) removed the glucose while a disaccharide mixture (18·1 g.), containing nigerose and maltose, was eluted with 7% aqueous ethanol (6·8 l.) and 10% aqueous ethanol (2·5 l.). Thereafter elution with 15% aqueous ethanol (20·5 l.) gave fractions containing trisaccharides (9·9 g.), tetrasaccharides (5·8 g.), and higher saccharides (8·8 g.).

Separation of the Disaccharide Fraction by Furanoside Formation.—The disaccharides (4 g.) were treated with 4% methanolic hydrogen chloride (180 c.c.) at room temperature for 74 min. During this time the solution showed $[\alpha]_D^{17}$ +130° (4 min.) → +103° (40 min.) → +101° (73 min.). The solution was neutralised with silver carbonate, the suspension centrifuged, and the methanol removed *in vacuo* over freshly washed barium carbonate below 35°. The syrup

[10] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.

was extracted with water (50 c.c.) and fractionated on a charcoal column (17 cm. × 9 cm.²). The charcoal had been previously washed with distilled water until the pH of the washings was the same as that of the distilled water, and then dialysed against running tap-water for 2 weeks. After the column had been washed with water (700 c.c.), 5% ethanol (1·4 l.) eluted the maltose (1·783 g.) while the methyl furanosides of nigerose were obtained (I) with 10% ethanol {1·1 l.; 0·576 g. having $[\alpha]_D^{17}$ +40° (*c* 0·48 in H_2O)} and (II) with 30% ethanol {800 c.c.; 0·703 g. having $[\alpha]_D^{17}$ +128° (*c* 0·49 in H_2O)}.

Examination of the Methyl Furanosides of Nigerose.—Both fractions (I) and (II) were non-reducing to the Shaffer–Hartmann reagent [7] and had OMe 8·2% (a methyl furanoside of nigerose requires OMe, 8·7%).

(i) *Lead tetra-acetate oxidation.* Fraction (II) (26·5 mg.) was dissolved in dry glacial acetic acid (25 c.c.), and a saturated, standardised solution of lead tetra-acetate in dry glacial acetic acid (4 c.c.) added. The solution was diluted to 50 c.c. with glacial acetic acid and kept at 25° in a flask which was wrapped in aluminium foil to exclude light. The uptake of lead tetra-acetate (moles per mole of furanoside) was 0·7 hr., 0·07; 1·7 hr., 0·15; 4·7 hr., 0·35; 10·5 hr., 0·57; 23·5 hr., 0·83; 47·5 hr., 1·06; 101 hr., 1·34; 192 hr., 1·63.

(ii) *Periodate oxidation.* Fraction (II) (53·3 mg.) was treated with 0·075M-sodium periodate (50 c.c.) in the dark at room temperature. The number of moles of sodium periodate consumed per mole of furanoside was: 5 min., 1·5; 15 min., 2·3; 60 min., 2·4; 120 min., 2·7; 1140 min., 3·1. After 1140 min., 1·0 mole of formic acid and 1·2 moles of formaldehyde (isolated as methylene-bisdimedone, m. p. and mixed m. p. 192°) were produced per mole of furanoside.

Fraction (I) when treated as above consumed 1·9 moles (5 min.), 2·5 moles (60 min.), 2·6 moles (120 min.), 3·0 moles (1110 min.) of periodate per mole of furanoside. After 1110 min., 1·1 moles of formic acid and 0·8 mole of formaldehyde (characterised as above) were produced per mole of furanoside.

(iii) *Conversion of the product of periodate oxidation into xylose.* Fraction (I) (259 mg.) was treated in the dark with sodium periodate (350 mg.) in water (100 c.c.), at room temperature for 36 hr. Excess of periodate was then destroyed by the addition of ethylene glycol (0·5 ml.), and the solution concentrated *in vacuo* below 30° to 25 c.c. Sodium borohydride (250 mg.) was added and the solution kept overnight at room temperature. Excess of borohydride was then destroyed by the addition of Amberlite IR-120. The resin was filtered off and the solution was made N with respect to sulphuric acid. After 2 hr. at 100° the solution was cooled and free iodine extracted from it with carbon tetrachloride. After neutralisation with barium carbonate the solution was clarified in the centrifuge and evaporated *in vacuo* to a syrup which was distilled three times with dry methanol in order to remove the methyl borate. This syrup was fractionated on a cellulose column (23 cm. × 8 cm.²) which was developed with butanol–ethanol–water–ammonia (40 : 16 : 20 : 1). Those fractions which only contained a component with the same R_F value as xylose were combined and concentrated to a syrup (33 mg.) having $[\alpha]_D$ +15° in water. On acetylation of the syrup with sodium acetate and acetic anhydride, crystals (7 mg.) were obtained having m. p. 124—125°, not depressed on admixture with β-tetra-*O*-acetyl-D-xylose.

(iv) *Methylation of the furanosides of nigerose.* A mixture of fractions (I) and (II) (550 mg.) was methylated in dioxan (25 c.c.) at 40° by alternate additions of dimethyl sulphate (20 c.c.) and 30% aqueous sodium hydroxide (40 c.c.) in ten equal amounts. After the resulting mixture had been stirred for 3 days at room temperature, water (50 c.c.) was added and the mixture heated at 90—95° for 30 min. The methylated product was extracted with chloroform (10 × 25 c.c.), and the syrup (442 mg.) obtained by concentrating the dried extract then submitted to three further methylations with Purdie reagents. A portion (2 mg.) of the product (0·362 g.) was hydrolysed and the products were separated on a paper chromatogram irrigated with the organic phase of butanol–ethanol–water–ammonia (40 : 10 : 49 : 1). Only two spots could be detected; they had R_F values (i) identical with that of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and (ii) somewhat less than that of 2 : 3 : 6-tri-*O*-methyl-D-glucose.

(v) *Separation and examination of the products from the hydrolysis of the methylated nigerose furanosides.* The methylated product (325 mg.) was refluxed with 4% methanolic hydrogen chloride (30 c.c.) for 4 hr. The solution was neutralised with silver carbonate, clarified in the centrifuge, and evaporated *in vacuo* to a syrup. These glycosides were then hydrolysed with 0·5N-HCl for 4 hr. at 100°. After the solution had been freed from ions as above the resulting methyl sugars (191 mg.) were fractionated on a silica column by Bell's method.[9]

Elution with chloroform gave a fraction (120 mg.) which behaved in a manner identical with that of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose when examined on a paper chromatogram irrigated as above. A second fraction (38 mg.) contained the unknown tri-*O*-methyl sugar which had an R_F value (0·62) different from that of 2 : 3 : 5-tri-*O*-methyl- (R_F 0·67), 2 : 3 : 6-tri-*O*-methyl- (R_F 0·66), and 2 : 3 : 4-tri-*O*-methyl-D-glucose (R_F 0·68). It had $[\alpha]_D$ +48° (*c* 0·68 in $CHCl_3$).

(vi) *Examination of the unknown tri-O-methyl sugar.* The tri-*O*-methyl-D-glucose fraction (34 mg.) was treated with 4% methanolic hydrogen chloride (6 c.c.) at room temperature. The solution had $[\alpha]_D^{17}$ +53° (4 min.) ⟶ +39° (32 min.) ⟶ +35° (67 min.). The tri-*O*-methyl sugar was regenerated by concentration to a syrup, hydrolysis with 0·1N-hydrochloric acid (10 c.c.) at 100° for 3 hr., and subsequent neutralisation with silver carbonate and concentration of the filtered solution *in vacuo*.

The tri-*O*-methyl sugar was dissolved in water (5 c.c.) and treated with aqueous sodium borohydride (60 mg. in 5 c.c.) at room temperature for 18 hr. After removal of ions as described above, the solution was concentrated to a non-reducing syrup (13·2 mg.). When submitted to periodate oxidation under the conditions described for fraction (I), the tri-*O*-methylhexitol consumed 0·3 mole (2·3 hr.), 0·7 mole (14·1 hr.), 0·9 mole (22·1 hr.), 0·9 mole (27·1 hr.) of periodate per mole of tri-*O*-methyl hexitol. After 27·1 hr., it produced 0·1 mole of formic acid per mole of tri-*O*-methylhexitol, but no formaldehyde.

(vii) *Partial acidic hydrolysis of the nigerose furanosides.* Fraction (I) (172 mg.) was treated with 0·01N-hydrochloric acid (172 c.c.) at 45—55°. The reducing power (expressed as mg. of glucose/2 c.c. of solution) of the solution was determined at intervals (see Table) by using the Shaffer–Hartmann reagent.[7]

Time (hr.)	5	10	27	32	37	50	57
Reducing power	0·26	0·44	0·60	0·63	0·64	0·65	0·65

At the same intervals, aliquot parts (2 c.c.) were withdrawn and analysed by paper ionophoresis in borate buffer.[5] This revealed the progressive formation of nigerose; no other reducing sugar was detected.

After 57 hr., the solution was neutralised with silver carbonate, clarified in the centrifuge, evaporated *in vacuo* to a small volume, and freeze-dried (120 mg.). The recovered nigerose showed $[\alpha]_D$ +137° (*c* 0·17 in H_2O).

The above experiment was repeated on fraction (II) on a smaller scale and gave similar results.

Separation of the Trisaccharide Fraction by Furanoside Formation.—The trisaccharide fraction contained two components which could be separated by ionophoresis [5] in borate buffer of pH 10. The slower-moving trisaccharide was designated T_1, and the faster-moving trisaccharide was designated T_2.

A trisaccharide mixture (6·701 g.) was treated with 4% methanolic hydrogen chloride (150 c.c.) at room temperature for 81 min. During this time the solution had $[\alpha]_D^{17}$ +161° (8 min.) ⟶ +141° (40 min.) ⟶ +139° (80 min.). The solution was neutralised with silver carbonate (30 g.), clarified in the centrifuge, and evaporated *in vacuo* at 30° over freshly washed barium carbonate, and the residue taken up in water and filtered. The solution was then passed down a charcoal column (16 cm. × 14 cm.^{2}). Elution with 13% ethanol (6 l.) gave chromatographically pure trisaccharide T_1 (1·646 g.) and later mixed fractions (0·879 g., 0·261 g., 0·640 g., 0·505 g.) in which the presumed furanosides of T_2 were accompanied by progressively decreasing concentrations (28, 8·3, 7·5, 7·3%) of T_1 when estimated by the Shaffer–Hartmann method [7] for reducing power. The major portion of these fractions was refractionated on cellulose columns with butanol–ethanol–water–ammonia (40 : 16 : 20 : 1). Distinct separations were achieved between the furanosides of T_2 and trisaccharide T_1, and these were recovered in yields of 1·452 g. and 0·210 g. respectively.

Examination of Trisaccharide T_1.—The trisaccharide was reducing, and 3 mg. of T_1 exhibited the same reducing power as 0·77 mg. of glucose (Shaffer–Hartmann method [7]). It had $[\alpha]_D$ +169·5° (*c* 1·25 in H_2O). Its infrared spectrum showed strong absorption at 917, 841, and 835 cm.$^{-1}$ (the last two are indicative of α-linkages) and moderate absorption at 796 and 774 cm.$^{-1}$. Hydrolysis of the trisaccharide (8·07 mg.) with 1·5N-sulphuric acid at 100° for 5 hr. gave glucose (identified on a paper chromatogram) in an amount corresponding to a 97·5% conversion.

(i) *Partial acidic hydrolysis.* The trisaccharide T_1 (1 g.) was submitted to partial acidic

hydrolysis with 0·3N-sulphuric acid (20 c.c.) at 85—90° for 45 min. After neutralisation with barium carbonate the solution was passed down a charcoal column. The glucose was removed by water-washing, and the mixture of disaccharides (0·093 g.) eluted with 8% aqueous ethanol. The nigerose and maltose mixture was separated by furanoside formation and then fractionated on a cellulose column as previously described. This yielded maltose (35 mg.) and the furanosides of nigerose (43 mg.). The maltose was characterised by conversion into its β-octa-acetate (12 mg.), m. p. and mixed m. p. 159—160°. The furanoside of nigerose was hydrolysed to nigerose (as above), and this was characterised by conversion into the osazone (23 mg.), m. p. and mixed m. p. 204—206°.

(ii) *Reduction to the sugar alcohol.* Trisaccharide T_1 (100 mg.) was treated with aqueous sodium borohydride (100 mg. in 50 c.c.) at room temperature for 3 hr. After removal of ions as described above, the trisaccharide alcohol (65 mg.) was recovered by freeze-drying.

Some (10 mg.) of the alcohol was partially hydrolysed with 0·3N-sulphuric acid (1 c.c.) at 90° for 45 min. After neutralisation with barium carbonate and filtration, paper chromatography (for reducing sugars) revealed glucose, nigerose, and a trace of T_1. No maltose could be detected.

The remainder (51·8 mg.) of the sugar alcohol of T_1 was oxidised with sodium periodate as described above. The number of moles of periodate consumed per mole of trisaccharide alcohol was 2·4 (0·25 hr.), 2·5 (0·5 hr.), 3·0 (1 hr.), 3·9 (4 hr.), 4·7 (18 hr.), 4·9 (23 hr.), 4·9 (28 hr.). The corresponding figures, after 28 hr., for formic acid and formaldehyde produced were 2·2 moles and 1·9 moles, respectively.

The solution from the oxidation was made N with respect to sulphuric acid and heated at 100° for 3 hr. Free iodine was extracted with carbon tetrachloride, and sodium ions were removed from the solution with Amberlite IR-120. The solution was then neutralised with barium carbonate, and clarified by centrifuging. Analysis on a paper chromatogram revealed glucose.

Examination of the Methyl Furanosides of Trisaccharide T_2.—The presumed furanosides were non-reducing to the Shaffer–Hartmann reagent [7] and had OMe 6·1% (a methyl furanoside of a trisaccharide requires OMe, 6·0%).

(i) *Periodate oxidation.* The furanosides (96·3 mg.) were oxidised with sodium periodate as described above. The number of mols. of periodate consumed per mol, of furanosides was 3·0 (0·25 hr.), 3·2 (0·5 hr.), 3·4 (1 hr.), 3·7 (4 hr.), 3·9 (18 hr.), and 3·9 (27 hr.). After 27 hr., 0·7 mole of formaldehyde and 1·2 moles of formic acid were produced per mole of furanoside.

(ii) *Lead tetra-acetate oxidation.* The furanosides of T_2 (24·9 mg.) were oxidised with lead tetra-acetate as described for the oxidation of the furanosides of nigerose :

Time (hr.)	1	15	22	88	136	232	287	321
Uptake of $Pb(OAc)_4$ (mols.)	0·12	1·03	1·20	2·13	2·61	3·28	3·51	3·59

(iii) *Conversion into trisaccharide T_2.* The furanosides (370 mg.) were heated in 0·01N-sulphuric acid at 50° for 5 days. Ionophoresis in borate buffer (pH 10·0) showed the presence of trisaccharide T_2 only and this was recovered by neutralising the solution with barium carbonate, then centrifuging and freeze-drying it to a powder (0·308 g.).

(iv) *Conversion into maltose and nigerose.* The furanosides of T_2 (900 mg.) were submitted to partial acidic hydrolysis, and the products separated as described for trisaccharide T_1. A mixture (87 mg.) of maltose and nigerose was obtained from which maltose (37 mg.) and the furanosides of nigerose (39 mg.) were isolated. The maltose was characterised as its β-octa-*O*-acetate (16 mg.), m. p. and mixed m. p. 159—160°. The furanosides of nigerose were hydrolysed to nigerose and this was characterised by conversion into the osazone (28 mg.), m. p. and mixed m. p. 203—206°.

Examination of Trisaccharide T_2.—Its infrared spectrum showed strong absorption at 917, 843, and 835 cm.$^{-1}$ (the last two are indicative of α-linkages) and moderate absorption at 797 and 770 cm.$^{-1}$. Hydrolysis of the trisaccharide (10·4 mg.) with 1·5N-sulphuric acid at 100° for 5 hr. gave glucose (identified on a paper chromatogram) in an amount corresponding to a 99·7% conversion.

(i) *Reduction to the sugar alcohol.* The trisaccharide T_2 (150 mg.) was dissolved in water (40 c.c.), and Raney nickel (0·5 g. in ethanol) was added. The mixture was heated and stirred in an autoclave at 140° with an initial hydrogen pressure of 100 atm. which rose to 135 atm. during the heating time (3 hr.). After cooling, the Raney nickel was filtered off and the solution

freeze-dried (136 mg.). The product was non-reducing to the Shaffer–Hartmann reagent [7] and moved as a single component on a paper chromatogram.

(ii) *Partial hydrolysis of the sugar alcohol.* Part (10 mg.) of the product was submitted to partial hydrolysis with 0·3N-sulphuric acid at 90° for 45 min. Paper chromatography of the neutralised hydrolysate showed that the only reducing sugars present were glucose and maltose.

(iii) *Periodate oxidation of the sugar alcohol.* The remainder (119·5 mg.) of the sugar alcohol was oxidised with sodium periodate as previously described. The consumption of periodate (moles per mole of trisaccharide alcohol) was 3·9 (1 hr.), 5·4 (7 hr.), 6·1 (21 hr.), 6·1 (24·5 hr.). After 24·5 hr., 2·2 mols. of formic acid and 1·9 mols. of formaldehyde were produced.

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CHEMISTRY DEPARTMENT, THE UNIVERSITY,
EDGBASTON, BIRMINGHAM, 15.

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