Structure of a Polysaccharide from the Seeds of *Cassia tora*. Part II.[1] Partial Acidic Hydrolysis

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Partial acidic hydrolysis of the polysaccharide obtained from the seeds of *Cassia tora* Linn. gave the following oligosaccharides, which were characterized on the basis of chemical and physical constants: 4-*O*-β-D-glucopyranosyl-D-mannopyranose (0.83%), 4-*O*-β-D-mannopyranosyl-D-mannopyranose (5.17%), 4-*O*-β-D-glucopyranosyl-D-glucopyranose (0.48%), 6-*O*-β-D-glucopyranosyl-D-glucopyranose (0.47%), 6-*O*-α-D-galactopyranosyl-D-mannopyranose (3.98%), *O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-D-glucopyranose (0.57%), *O*-α-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (1.05%), *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose (0.70%), *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-mannopyranosyl(1→4)-D-mannopyranose (0.78%), *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (0.52%), *O*-α-D-galactopyranosyl-(1→6)-*O*-[β-D-mannopyranosyl-(1→4)]-D-mannopyranose (2.58%), *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose (1.50%), *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (0.63%), *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose (7.08%), *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose (5.25%), *O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose (2.33%), *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (0.67%), *O*-β-D-glucopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_3$→4)-D-glucopyranose (0.79%), *O*-β-D-glucopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_4$→4)-D-glucopyranose (0.92%), and *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_3$→4)-D-glucopyranose (2.46%). Efforts have also been made to ascertain the pattern of hydrolysis from studies of degraded polysaccharides isolated at various intervals during partial acidic hydrolysis.

THE polysaccharide from the defatted seeds of *Cassia tora* Linn. (Leguminosae) was obtained and purified by the usual method.[1-4] The purified polysaccharide ($[\alpha]_D^{27}$ +34.5° in N-NaOH) was found to be homogeneous [1] by fractional precipitation followed by hydrolysis and quantitative estimation of the component sugars in each fraction, zone electrophoresis, and acetylation followed by deacetylation. Quantitative estimation [1] showed that D-galactopyranose, D-glucopyranose, D-mannopyranose, and D-xylopyranose units were present in the molar ratio 2 : 2 : 7 : 1. The methylated polysaccharide yielded [1] 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3-di-*O*-methyl-D-mannose, and 2,3-di-*O*-methyl-D-glucose in the approximate molar ratios 1 : 4 : 8 : 1 : 1 : 20 : 8 : 5. Graded hydrolysis indicated that D-xylose was liberated first, followed by D-galactose, D-glucose, and ultimately D-mannose. We now report the results of partial acidic hydrolysis of the polysaccharide.

The structures of various oligosaccharides isolated during partial acidic hydrolysis were assigned on the basis of complete acidic hydrolysis followed by chromatographic examination, equivalent weight determination by the alkaline hypoiodite method,[5] enzymic hydrolysis followed by paper chromatographic examination, oxidation by periodate, partial acidic hydrolysis, methylation by Kuhn's procedure followed by methanolysis and subsequent hydrolysis, measurements of specific optical rotation, and plotting of $[M]_D$ and log $[R_{Glc}/(1 - R_{Glc})]$ against degree of polymerisation (DP). French and Wild [6] have established a linear relationship between log $[R_F/(1 - R_F)]$ values of oligosaccharides of a homologous series and their DPs. Similar results were obtained by Aspinall *et al.*,[7] who plotted R_M values against DP [R_M = log $(1/R_F - 1)$]. Recently R_F has been replaced by either R_{Glc} [8] or R_{Gal} [9] with advantage, particularly when R_F values of oligomers are very low.

The products from the partial acidic hydrolysis of the polysaccharide are listed in Table 1.

The oligosaccharides (k), (p), and (t), and also *O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-(D-mannopyranose(u), *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (v), and *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (w) have not been reported previously. The oligosaccharide (u) was obtained from the partial acidic hydrolysis of (p) and the other two [(v) and (w)] were obtained from that of (t). The structures of the oligosaccharides (k), (p), and (t) were assigned on the basis of partial acidic hydrolysis and methylation studies. In the cases of the oligosaccharides (f), (h), (i), (j), and (l), the m.p.s are reported here for the first time.

From the studies of degraded polysaccharides (P_1 to

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P_5), obtained after hydrolysis for ½, 1, 2, 3, and 4½ h, information regarding the anomeric configurations of the component sugar units as well as the mode of breaking of their linkages during partial acidic hydrolysis have been

TABLE 1

Yields (%) of products isolated from partial acidic hydrolysis of the polysaccharide from *C. tora* seeds

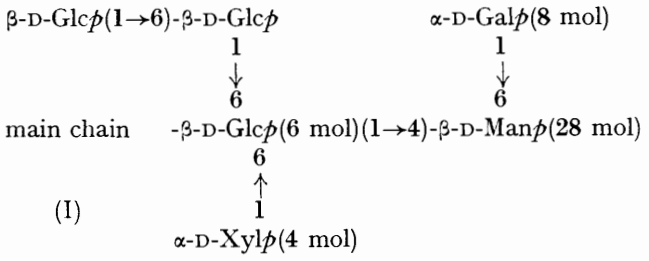
Monosaccharides (41.67)		
D-Galactose		9.00
D-Glucose		3.08
D-Mannose		22.92
D-Xylose		6.67
Disaccharides (10.93)		
(a)	4-*O*-β-D-Glucopyranosyl-D-mannopyranose (1) *	0.83
(b)	4-*O*-β-D-Mannopyranosyl-D-mannopyranose (2)	5.17
(c)	4-*O*-β-D-Glucopyranosyl-D-glucopyranose (X_1)	0.48
(d)	6-*O*-β-D-Glucopyranosyl-D-glucopyranose (3)	0.47
(e)	6-*O*-α-D-Galactopyranosyl-D-mannopyranose (5)	3.98
Trisaccharides (20.66)		
(f)	*O*-β-D-Glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-D-glucopyranose (4)	0.57
(g)	*O*-α-D-Xylopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (6)	1.05
(h)	*O*-β-D-Glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose (X_2)	0.70
(i)	*O*-β-D-Glucopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose (X_3)	0.78
(j)	*O*-β-D-Mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (X_4)	0.52
(k)	*O*-α-D-Galactopyranosyl-(1→6)-*O*-[β-D-mannopyranosyl-(1→4)]-D-mannopyranose (X_5)	2.58
(l)	*O*-β-D-Mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose (X_6)	1.50
(m)	*O*-β-D-Glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (10)	0.63
(n)	*O*-β-D-Mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose (11)	7.08
(o)	*O*-α-D-Galactopyranosyl-(1→6)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose (12)	5.25
Tetrasaccharides (3.00)		
(p)	*O*-β-D-Glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose (X_7)	2.33
(q)	*O*-β-D-Glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl (1→4)-D-glucopyranose (Y_1)	0.67
Pentasaccharides (0.79)		
(r)	*O*-β-D-Glucopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_3$→4)-D-glucopyranose (Y_2)	0.79
Hexasaccharides (3.38)		
(s)	*O*-β-D-Glucopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_4$→4)-D-glucopyranose (Y_3)	0.92
(t)	*O*-β-D-Mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_3$→4)-D-glucopyranose(Y_4)	2.46
Degraded polysaccharides (P_1—P_5)		9.17

* Fraction numbers in parentheses.

derived. Quantitative estimations of the component sugars of these degraded polysaccharides showed that D-xylopyranose units followed by D-galactopyranose units, were preferentially hydrolysed during the first 2 h and then the linkages between D-glucopyranose and D-mannopyranose units. This is evident from the compositions of the degraded polysaccharides P_1 to P_5 which contained xylose in decreasing order while galactose decreased from P_1 to P_3 and then rapidly increased in P_4 and P_5. The molar ratio between galactose and mannose units in P_5 has been found to be 1 : 3, which is close to that in the original polysaccharide (1 : 3.5).[1] Although the galactose units were more easily hydrolysed than glucose and mannose units of the main chain, the unbranched portion formed by the removal of galactose units was comparatively more susceptible towards acidic hydrolysis than the branched portion of the degraded polysaccharide containing galactose as single unit branches, and therefore the proportion of galactose increased in the degraded polysaccharides. Specific rotations of the degraded polysaccharides (P_1 to P_5) are plotted against time at which they were obtained from the hydrolysis mixture in Figure 3. The positive optical rotation decreased gradually till the degraded polysaccharide acquired a negative rotation at 2 h and then increased reaching almost the original value for the polysaccharide at 4½ h. These changes in optical rotation suggest that D-xylopyranose and D-galactopyranose units have α-anomeric configurations, because their hydrolysis decreases the positive optical rotation, whereas D-glucopyranose and D-mannopyranose units in the polysaccharide have β-configurations, as their hydrolysis results in an increase.

From the above results, the following information can be derived. The main chain consists of β-(1→4)-linked D-glucopyranose and D-mannopyranose units; D-galactopyranose units form single unit branches attached to D-mannopyranose units only by α-(1→6)-linkages; D-xylopyranose units are present as single unit branches attached to β-(1→4)-linked D-glucopyranose units by α-(1→6)-linkages; β-(1→6)-linked D-glucopyranose units are present in the branches only, each containing an *O*-β-D-glucopyranosyl-(1→6)-D-glucopyranosyl unit attached to a β-(1→4)-linked D-glucopyranose unit of the main chain by a β-(1→6)-linkage; and D-glucopyranose units, linked to *O*-β-D-glucopyranosyl-(1→6)-D-glucopyranose units by β-(1→6)-linkages, are in turn attached to D-mannopyranose units by β-(1→4)-linkages. The major products, 4-*O*-β-D-mannopyranosyl-D-mannopyranose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose, 6-*O*-α-D-galactopyranosyl-D-mannopyranose, *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose, and *O*-α-D-galactopyranosyl-(1→6)-*O*-[β-D-mannopyranosyl-(1→4)]-D-mannopyranose were obtained in a total yield of 24.1%, showing the presence of extensive units of *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose or *O*-β-D-mannopyranosyl-(1→4)-*O*-[α-D-galactopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose joined together through D-mannopyranose units by β-(1→4)-linkages forming a long chain. Both the above units on partial acidic hydrolysis would be expected to give galactose, mannose, 4-*O*-β-D-mannopyranosyl-D-mannopyranose, 6-*O*-α-D-galactopyranosyl-D-mannopyranose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose, *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose, and *O*-α-D-galactopyranosyl-(1→6)-*O*-[β-D-mannopyranosyl-(1→4)]-D-mannopyranose.

Thus, on the basis of the results described so far, the generalised structure (I) can be assigned to a repeating unit of the polysaccharide from the seeds of *Cassia tora* Linn. The structure contains 48 sugar units; it fully explains the formation of the oligosaccharides obtained from the partial acidic hydrolysis and completely agrees with the analytical data.



(I)

A polysaccharide such as (I) should consume during periodate oxidation 62 mol. equiv. of periodate and liberate 14 mol. equiv. of formic acid per repeating unit of 48 sugar residues. The actual consumption of periodate and liberation of formic acid found were 63.2 and

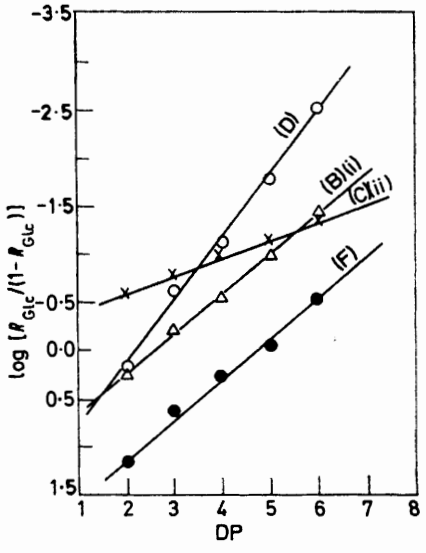


FIGURE 1 Plots of $\log[R_{Glc}/(1 - R_{Glc})]$ (solvents as indicated) for cellodextrins against the degree of polymerisation (DP)

14.3 mol. equiv., respectively, per repeating unit of the polysaccharide. I.r. spectral studies [10] also indicated the existence of α- and β-linkages by the characteristic absorption bands at 820, 880, and 785 cm^{-1}.

EXPERIMENTAL

The isolation, purification, and determination of homogeneity of the polysaccharide are described in Part I.[1] Solutions were evaporated under reduced pressure at 40 °C or below, and residues dried *in vacuo* at room temperature over anhydrous calcium chloride. Unless otherwise stated, specific rotations are equilibrium values, and were measured for aqueous solutions at *ca.* 32 °C. Paper chromatographic separations were carried out at room temperature (25—35 °C) by the descending technique on Whatman Nos. 1 and 3 MM papers with the organic phase of any one of the following solvent systems (v/v): (A) butan-1-ol–propan-2-ol–water (11 : 6 : 3);[3,9] (B) ethyl acetate–pyridine–water (i) (2 : 1 : 2),[11] (ii) (5 : 2 : 5),[12] and (iii) (10 : 4 : 3);[13] (C) butan-1-ol–pyridine–water (i) (6 : 4 : 3)[12] and (ii) (5 : 3 : 2);[14] (D) butan-1-ol–pyridine–water–benzene (5 : 3 : 3 : 1);[15] (E) propan-1-ol–ethyl acetate–water (7 : 1 : 2);[12] (F) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4);[16] (G) butan-1-ol–ethanol–water (5 : 1 : 4);[17] (H) butan-2-one–

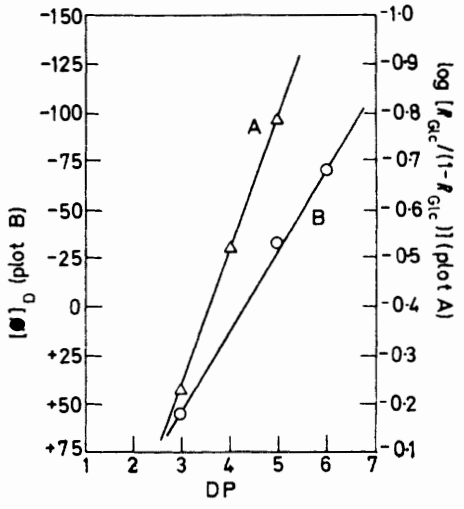


FIGURE 2 Plots of $\log[R_{Glc}/(1 - R_{Glc})]$ and $[M]_D$ for *O*-β-D-mannopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_n$→4)-D-glucopyranoses against the degree of polymerisation (DP)

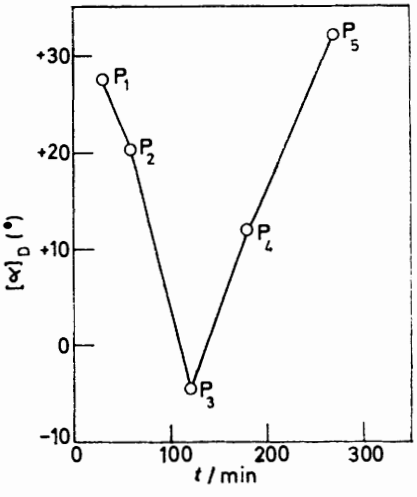


FIGURE 3 Plot of specific rotations of degraded polysaccharides (P_1 to P_5) against time at which they were taken from the hydrolysis mixture

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water (10 : 1);[18] (I) butan-1-ol–acetic acid–water (4 : 1 : 5); (J) butan-2-one–water–ammonia (100 : 50 : 3); and (K) benzene–ethanol–water (169 : 47 : 15).[19] Sugar spots were visualised by spraying with aniline hydrogen phthalate in moist butan-1-ol and heating at 110—120 °C in an electric oven for 15—20 min. A general description of the techniques used for the structure elucidation of oligosaccharides is given below. For quantitative analyses the substances were weighed to the nearest 0.01 mg. Micropipettes (1 ml) and microburettes (10 ml) used were graduated in units of 0.01 ml.

Identification of Component Sugars.—The oligosaccharide (*ca.* 5 mg) was hydrolysed with 1.5N-sulphuric acid (1 ml) in a micro-flask fitted with an air condenser on a boiling water-bath for 15 h; the product was diluted to 5 ml with water, neutralised ($BaCO_3$), filtered, and concentrated. The sugars in the hydrolysate were identified by paper co-chromatography in any one of the solvents (A), (G), and (I), and also by co-t.l.c. on silica gel G layers buffered with boric acid [benzene–acetic acid–methanol (1 : 1 : 3) and butan-2-one–acetic acid–methanol (3 : 1 : 1) as solvent systems [20]] with authentic sugars.

Quantitative Estimation of Component Sugars.—Each of the oligosaccharides (e), (k), and (o) (10—15 mg) was hydrolysed under reflux at 100 °C for 12 h, and D-ribose (3—5 mg) was added. The hydrolysate was diluted to 15 ml, neutralised ($BaCO_3$), filtered, and concentrated. The monosaccharides in the hydrolysate were separated chromatographically in solvent (A) on three sheets (12 × 22 in, Whatman No. 1) each having three guide strips, two on the edges and one in the centre. Concurrently, three similar sheets as blanks were developed with the same solvent. The guide strips were cut from the developed chromatogram and the sugar spots located. With the help of these guide strips, appropriate sections were cut from the unsprayed portions of each chromatogram containing sugar spots. Sections of the same dimension and location corresponding to those containing sugars were cut from the blank chromatograms. Each section, containing a sugar, and the corresponding paper blank was cut into pieces and extracted separately in micro-Soxhlet extractors with methanol (20 ml; B. D. H. AnalaR) for 1 h. The solvent was removed from each extract, and the sugar residue was dissolved in water (10 ml), and assayed by the periodate oxidation method of Hirst and Jones.[21]

In another method, which was used more frequently, the oligosaccharide (5—7 mg) was hydrolysed with 1.5N-sulphuric acid (1 ml) as above, and D-ribose [1.5—2.0 mg (2 ml of a freshly prepared standard solution)] was added. After dilution to 10 ml with water, the hydrolysate was neutralised ($BaCO_3$), filtered, concentrated, and separated on three sheets (8 × 22 in) of Whatman No. 1 paper in solvent (A). Three paper blanks of the same size were also developed with the same solvent under identical conditions. Three guide strips were cut from each chromatogram as described above, and sprayed with aniline hydrogen phthalate, and the sugar spots were located. After reassembling the guide strips and unsprayed portions of the chromatogram, the best lines of demarcation were drawn between the spots, and sections were cut out. The paper blank was also cut into sections corresponding to the area and location of the sugars on the first chromatogram. The sections were transferred to separate beakers, and distilled water (10 ml) was added to each beaker, which was then covered and set aside for 30 min with occasional shaking; the extracts were then filtered through glass wool. Portions (2 ml) in triplicate of each extract were treated with aqueous 5% phenol (1 ml) and sulphuric acid (B.D.H. AnalaR; *d* 1.84; 5 ml).[22] The absorbance of the characteristic yellow orange colour produced was measured with a Klett-Summerson photoelectric colourimeter [using filter No. 50 against a blank, prepared with water (2 ml) and phenol–sulphuric acid reagent]. From the absorbance data, the amounts of sugars separated chromatographically were calculated with the help of their calibration curves prepared under identical conditions.

In both the above procedures, recoveries of the component sugars of oligosaccharides were calculated on the basis of complete recovery of D-ribose.

Permethylation.—The oligosaccharides (10—20 mg) were permethylated according to the Kuhn procedure [23,24] with methyl iodide and silver oxide in dimethylformamide. The permethylated oligosaccharides (*ca.* 4 mg) were hydrolysed [25] at 100 °C with 90% formic acid (2 drops) for 4 h, and then with N-sulphuric acid (1 ml) for 6 h, and the hydrolysate was examined by paper chromatography in any two of the solvents (G), (H), (J), and (K). Mobilities of the methylated sugars relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose (R_{TMG}) were calculated and compared with those given in the literature. Final identification was made by co-chromatography on paper and t.l.c.[20] with authentic samples of methylated sugars.[1]

Quantitative Examination of Methylated Sugars.—The methylated oligosaccharide (3—6 mg) was heated in methanolic 4% hydrogen chloride (2 ml) under reflux for 4 h, and D-glucose [1—2 mg (2 ml of a freshly prepared standard solution)] was added. The solvent was evaporated off under reduced pressure. Traces of hydrogen chloride were removed *in vacuo* over sodium hydroxide pellets. The residue so obtained was hydrolysed at 100 °C with 1.6N-sulphuric acid (1 ml) for 8 h, diluted to 10 ml with water, neutralised ($BaCO_3$), filtered, and concentrated. By a procedure similar to that described above for the quantitative spectrophotometric estimation of component sugars, the hydrolysate was separated on paper (two sheets) in solvent (J), and the methylated sugars were eluted with water and treated with phenol–sulphuric acid reagent.[22,26] The absorbance of the colour produced was measured at 485 nm on a Beckman DU spectrophotometer. The amounts of sugars were calculated from the corrected absorbances with the help of calibration curves of the sugars. Recoveries of methylated sugars were calculated on the basis of complete recovery of D-glucose.

Determination of Equivalent Weight.—The equivalent weight of an oligosaccharide (*ca.* 3 mg) was determined by the alkaline hypoiodite method;[5] 0.1N-iodine (1 ml) was used, with 2 ml of a solution 0.2M in sodium hydrogen carbonate and 0.2M in sodium carbonate. The reaction was

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carried out in a micro-iodine flask (5 ml) in the dark for 3 h, and the contents were transferred quantitatively to another flask (50 ml). After careful acidification, the unchanged iodine was titrated against 0.005N-sodium thiosulphate. The determinations were performed in duplicate.

TABLE 2

Results of periodate oxidation and enzymic hydrolysis of known oligosaccharides isolated from partial acidic hydrolysis of the polysaccharide

Fraction no.	Periodate oxidation: Periodate consumed (mol. equiv.)	Periodate oxidation: Formic acid liberated (mol. equiv.)	Mol. wt.	Hydrolysis with emulsin	Oligosaccharide identified
1	4.88	2.98	357.2	Glucose and mannose	(a)
2	5.10	3.13	347.6	Mannose	(b)
3	6.15	5.20	346.8	Glucose	(d)
4	7.85	5.87	514.2	Glucose	(f)
5	6.12	4.94	348.2	No hydrolysis	(e)
6	7.28	4.23	487.8	Glucose and isoprimeverose	(g)
X_1	4.80	3.20	347.0	Glucose	(c)
X_2	5.93	3.21	529.6	Glucose and mannose	(h)
X_3	5.80	3.00	526.0	Glucose and mannose	(i)
X_4	6.33	3.14	521.6	Glucose and mannose	(j)
X_6	5.81	3.30	528.0	Glucose and mannose	(l)
10	6.31	3.13	515.6	Glucose	(m)
11	5.94	3.07	547.0	Mannose	(n)
12	7.15	4.07	526.0	Galactose, mannose, and epimelibiose	(o)
Y_1	6.70	3.26	686.4	Glucose	(q)
Y_2	7.95	3.16	844.4	Glucose	(r)
Y_3	8.80	3.18	1 010.8	Glucose	(s)

Periodate Oxidation.—The oligosaccharide (10—15 mg) was dissolved in water, 0.2M-sodium periodate (5 ml) was added, and the volume was made up to 10 ml with water. A blank was prepared by diluting 5 ml of the same sodium periodate solution to 10 ml with water. The oxidation was conducted in the dark at 30 ± 2 °C. Samples (1 ml) were drawn in duplicate from each mixture and the blank at 24, 48, 72, and 96 h, and analysed separately for formic acid liberated and periodate consumed. The formic acid was determined by titration with 5×10^{-3}N-sodium hydroxide (Methyl Red; 1 drop of 0.01M-solution) after reduction of the excess of periodate by ethylene glycol (1 drop). The colour at the end-point was compared with that of the blank experiment. Periodate was determined by titrating the iodine liberated on adding sulphuric acid (1 ml; 2N) and 10% potassium iodide solution (2 ml) to the sample with 0.01N-sodium thiosulphate (starch; 2 drops of 0.1% solution). The titration was performed in a stoppered flask. The results are given in Table 2. After 96 h, the remaining reaction mixture was treated with 2N-sulphuric acid (2 ml) and 10% potassium iodide solution (4 ml), and the liberated iodine was titrated against N-sodium thiosulphate without indicator. The solution was concentrated to 5 ml, hydrolysed with 2N-sulphuric acid (5 ml) for 6 h, neutralised ($BaCO_3$), and filtered. The filtrate was deionised by passing through Amberlite IR-120 (H^+) and IR-4B resins, concentrated, and examined by paper chromatography. No sugar was detected in any case.

Partial Acid-Hydrolysis.—Unless otherwise stated, this was carried out with 0.1N-hydrochloric acid at 80 ± 5 °C for 20 min, with a 2% solution of the oligosaccharide.*

Enzymic Hydrolysis.—The oligosaccharide (*ca.* 6—10 mg) was suspended in an emulsion solution (5 ml), and kept at *ca.* 45 °C for 8 h. The solution was concentrated and examined by paper chromatography with authentic sugars. The results are given in Table 2.

Partial Acid-Hydrolysis of the Polysaccharide.—The powdered polysaccharide (12 g) was suspended in distilled water (600 ml) in a three-necked flask and stirred for *ca.* 12 h at 80 ± 3 °C to obtain a clear solution. The hydrolysis was carried out for 4½ h with continuous stirring by addition of hydrochloric acid (25.6 ml of conc. hydrochloric acid diluted to 80 ml) in portions. A sample (*ca.* 25 ml) of the mixture was taken out after ½, 1, 2, and 3 h, and the remaining portion was removed after 4½ h. These were marked as fractions I—V. The partly degraded polysaccharide was precipitated from each fraction with ethanol, filtered off, purified, dried *in vacuo*, and marked P_1 to P_5. The filtrates (alcohol supernatants) were neutralised with silver carbonate, filtered, concentrated, and marked F_1 to F_5. Paper chromatography revealed that syrups F_2 to F_5 contained the same number of oligosaccharides and they were therefore combined. The chromatogram of syrup F_1 showed the presence of four original monosaccharides and an unresolved streak. The results of physical and chemical examinations of P_1 to P_5 are given in Table 3.

TABLE 3

Examination of degraded polysaccharides

Degraded polysaccharide	Yield (mg)	$[\alpha]_D^{28}$ (°) (1N-NaOH)	Colour	Solubility	Acid hydrolysis	Molar ratio
P_1	300	+27.5	White	Soluble in hot water, dil. and conc. alkali	Gal, Glc, Man, and Xyl	5 : 6 : 22 : 2
P_2	185	+20.8	Light grey	Soluble in conc. alkali		6 : 10 : 21 : 1.75
P_3	160	−4.6	Grey			7 : 13 : 54 : 1.78
P_4	105	+12.0	Grey			3 : 4 : 14.8 : 0.32
P_5	350	+32.0	Grey		Gal, Glc, Man, and Xyl (traces)	11 : 12 : 13 (Xyl not estimated)

Examination of Hydrolysate (F_2 *to* F_5).—The combined syrup (10.8 g) dissolved in water (25 ml) was chromatographically separated on Whatman No. 3 MM papers in solvent (A) for 7 days. In all, 14 fractions were obtained by

* The oligosaccharides formed as a result of the partial acidic hydrolysis of the parent oligosaccharide were identified, wherever possible, by paper co-chromatography with authentic samples.

the usual method (numbered 1—14). Fractions 7—9 and 13 and 14 were not completely resolved. They were mixed together separately to form fractions X and Y, respectively. Separation of X in solvent (B) (i) on Whatman No. 3 MM papers yielded seven fractions (X_1 to X_7); that of Y in solvent (A) gave four fractions (Y_1 to Y_4).

Fraction 1: *oligosaccharide* (a). The sugar (100 mg) was crystallised from methanol; m.p. 134—135°, $[\alpha]_D$ +5.9° (*c* 1.0), equiv. wt. 178.6, R_{Man} 0.40 in solvent (A), R_{Glc} 0.88 in solvent (B) (i), $R_{cellobiose}$ 1.22 and 1.18 in solvents (B)(ii) and (C)(i), respectively. It formed an α-acetate, m.p. 200°, and a β-acetate, m.p. 162—163°. Acidic hydrolysis gave D-glucose and D-mannose (1 : 1).

Fraction 2: *oligosaccharide* (b). The sugar (620 mg), R_{Man} 0.34 in solvent (B)(ii), R_{Glc} 0.67 and 0.53 in (B)(i) and (B)(iii), was crystallised from methanol; m.p. 202°, $[\alpha]_D$ −8.9° (*c* 2.8). Acidic hydrolysis yielded D-mannose only. The oligosaccharide had equiv. wt., 173.8, and formed an osazone, m.p. 202—204°.

Fraction 3: *oligosaccharide* (d). The oligosaccharide (56 mg), R_{Man} 0.30 in solvent (A), R_{Glc} 0.50 in solvent (D), had m.p. 192° (dried), $[\alpha]_D$ +9.3° (*c* 0.50), equiv. wt. 173.4. Acidic hydrolysis gave D-glucose only.

Fraction 4: *oligosaccharide* (f). The sugar (68 mg), R_{Man} 0.27 in solvent (A), R_{Glc} 0.14 in solvent (D), was crystallised as an amorphous powder from ethanol; m.p. 188—196° (monohydrate), $[\alpha]_D$ −7.8° (*c* 0.60), equiv. wt. 257.1. It formed a β-acetate, m.p. 212°. Acidic hydrolysis yielded D-glucose only; partial hydrolysis with 0.1N-sulphuric acid for 20 min at 80 ± 5 °C gave glucose, 6-*O*-β-D-glucopyranosyl-D-glucopyranose (gentiobiose), and some unhydrolysed trisaccharide (Found: C, 41.25; H, 6.65. Calc. for $C_{18}H_{32}O_{16},H_2O$: C, 41.4; H, 6.5%).

Fraction 5: *oligosaccharide* (e). The oligosaccharide (478 mg), R_{Man} 0.23 in solvent (A), R_{Glc} 0.60 in solvent (B)(i), was crystallised from ethanol; m.p. 200°, $[\alpha]_D$ +122.5° (*c* 1.4), equiv. wt. 174; it formed an osazone, m.p. 172—175°. Acidic hydrolysis yielded D-galactose and D-mannose (1 : 1).

Fraction 6: *oligosaccharide* (g). The sugar (126 mg) showed R_{Man} 0.21 in solvent (A), and $R_{cellobiose}$ 0.52 and 0.81 in solvents (B)(ii) and (C)(i), respectively. It was crystallised from aqueous ethanol; m.p. 144—145°, $[\alpha]_D$ +158.2° (*c* 0.56), equiv. wt. 258.6. Acidic hydrolysis gave D-glucose and D-xylose (2 : 1); partial hydrolysis with 0.2N-hydrochloric acid for 20 min at *ca.* 80 °C yielded xylose, glucose, and 4-*O*-β-D-glucopyranosyl-D-glucopyranose (cellobiose). Enzymic hydrolysis with emulsin gave glucose and another sugar identified as 6-*O*-α-D-xylopyranosyl-D-glucopyranose (isoprimeverose), a hygroscopic solid, m.p. 200—202° (dried), $[\alpha]_D$ +130° (*c* 0.40). Thus the trisaccharide was identified as *O*-α-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (Found: C, 39.7. H, 7.0. Calc. for $C_{17}H_{30}O_{15},2H_2O$: C, 40.0; H, 6.65%).

Fraction X_1: *oligosaccharide* (c). The sugar (58 mg) had R_{Glc} 0.40 and 0.60 in solvents (B)(i) and (D), respectively and $R_{cellobiose}$ 1.00 in solvent (C)(i). It was crystallised from ethanol; m.p. 222°, $[\alpha]_D$ +33.4° (*c* 0.40), equiv. wt. 173.5; and formed an α-acetate, m.p. 226°, and a β-acetate, m.p. 200°. Acidic hydrolysis gave D-glucose only.

Fraction X_2: *oligosaccharide* (h). The sugar (84 mg), R_{Glc} 0.54 and 0.30 in solvents (B)(i) and (B)(iii), respectively, was crystallised from aqueous ethanol; m.p. 158—160° (monohydrate), $[\alpha]_D$ −3.4° (*c* 0.81), and equiv. wt. 264.8. Acidic hydrolysis yielded D-glucose and D-mannose (2 : 1); partial acidic hydrolysis yielded two more sugars. The first (16 mg), m.p. 224° (anhydrous), R_{Glc} 0.62 in solvent (B)(i), equiv. wt. 175.2, on acidic hydrolysis yielded D-glucose only; it was therefore identified as 4-*O*-β-D-glucopyranosyl-D-glucopyranose (cellobiose). The second (11 mg), having R_{Glc} and $R_{cellobiose}$ 0.86 and 1.15 in solvents (B)(i) and (C)(i), respectively, m.p. 134° (dried), equiv. wt. 182, gave on acidic hydrolysis D-glucose and D-mannose (1 : 1); it was therefore identified as 4-*O*-β-D-glucopyranosyl-D-mannopyranose (epicellobiose).

Fraction X_3: *oligosaccharide* (i). The sugar (94 mg), R_{Glc} 0.45 and 0.25 in solvents (B)(i) and (B)(iii), respectively, $R_{cellobiose}$ 0.62 and 0.64 in solvents (C)(i) and (E), respectively, was crystallised from aqueous ethanol; m.p. 148—152° (monohydrate), $[\alpha]_D$ −10.8° (*c* 0.98), equiv. wt. 263. Acidic hydrolysis gave D-glucose and D-mannose (1 : 2); partial acidic hydrolysis (0.2N-HCl; 15 min at 80 ± 5 °C) yielded two more sugars. The first, R_{Glc} 0.65 in solvent (B)(i), m.p. 196—200° (dried), gave D-mannose only on acidic hydrolysis. Thus it was identified as 4-*O*-β-D-mannopyranosyl-D-mannopyranose. The second had R_{Glc} 0.88 in solvent (B)(i), and m.p. 130—134° (dried); on acidic hydrolysis it gave D-glucose and D-mannose (1 : 1), and was therefore identified as 4-*O*-β-D-glucopyranosyl-D-mannopyranose (epicellobiose).

Fraction X_4: *oligosaccharide* (j). The oligosaccharide (62 mg), R_{Glc} 0.38 in solvent (B)(i), $R_{cellobiose}$ 0.26 and 0.40 in solvents (B)(ii) and (C)(i), respectively, was crystallised from aqueous ethanol; m.p. 166—168° (monohydrate), $[\alpha]_D$ +10.6° (*c* 0.60), equiv. wt. 260.8. Acidic hydrolysis gave D-glucose and D-mannose (2 : 1); partial acidic hydrolysis (0.15N-HCl; 15 min at 80 ± 5 °C) yielded two more sugars. The first had R_{Glc} 0.50 in solvent (B)(i) and m.p. 186—192° (uncrystallised); its acidic hydrolysis gave D-glucose and D-mannose (1 : 1), and thus it was identified as 4-*O*-β-D-mannopyranosyl-D-glucopyranose. The second, R_{Glc} 0.59 in solvent (B)(i), m.p. 212—218° (uncrystallised), on acidic hydrolysis yielded D-glucose only, and it was therefore identified as 4-*O*-β-D-glucopyranosyl-D-glucopyranose (cellobiose).

Fraction X_5: *oligosaccharide* (k). The oligosaccharide (315 mg), R_{Man} 0.11 in solvent (A), R_{Glc} 0.36 in solvent (B)(i), was crystallised from methanol; m.p. 182—184°, $[\alpha]_D$ +119° (*c* 1.20), equiv. wt. 265.7. Acidic hydrolysis gave D-galactose and D-mannose (1 : 2); acidic hydrolysis yielded 4-*O*-β-D-mannopyranosyl-D-mannopyranose and 6-*O*-α-D-galactopyranosyl-D-mannopyranose (epimelibiose) besides the monosaccharides. A periodate oxidation study showed the consumption of 5.88 mol of periodate and liberation of 4.34 mol of formic acid per mol (531.4 g) of (k). Enzymic hydrolysis with emulsin gave mannose and epimelibiose. Methylation of the oligosaccharide (15 mg) by the Kuhn method,[23,24] followed by hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,3,4,6-tetra-*O*-methyl-D-galactose, and 2,3-di-*O*-methyl-D-mannose (1 : 1 : 1), which indicated that one unit each of D-galactopyranose and D-mannopyranose is attached to the same D-mannopyranose unit. The oligosaccharide (k) has thus been identified as O-α-D-*galactopyranosyl*-(1→6)-O-[β-D-*mannopyranosyl*-(1→4)]-D-*mannopyranose* (most probably a sesquihydrate) (Found: C, 40.55; H, 6.7%; equiv. wt. 265.7. $C_{18}H_{32}O_{16},1.5H_2O$ requires C, 40.7; H, 6.6%; equiv. wt. 265.5).

Fraction X_6: *oligosaccharide* (l). The oligosaccharide (180 mg), R_{Glc} 0.29 in solvent (B)(i), $R_{cellobiose}$ 0.81 in solvent (E), was crystallised from ethanol; m.p. 190—196°

(monohydrate) and 250—255° (anhydrous), $[\alpha]_D$ −5.8° (*c* 1.10), equiv. wt. 264. Acidic hydrolysis gave D-glucose and D-mannose (1 : 2), but partial acidic hydrolysis yielded two disaccharides. The first, R_{Glc} and R_{Man} 0.49 and 0.40, respectively, in solvent (C)(ii), m.p. 198°, $[\alpha]_D$ +22.8° (*c* 0.53) on acidic hydrolysis gave D-glucose and D-mannose (1 : 1); it was thus identified as 4-*O*-β-D-mannopyranosyl-D-glucopyranose. The second, R_{Glc} and R_{Man} 0.66 and 0.53, respectively, in solvent (C)(ii), m.p. 201°, $[\alpha]_D$ −9.1° (*c* 0.88) on acidic hydrolysis gave D-mannose only, and it was therefore identified as *O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose.

Fraction X_7: *oligosaccharide* (p). The oligosaccharide (280 mg), R_{Glc} 0.15 and 0.26 in solvents (A) and (B)(i), respectively, was crystallised from aqueous ethanol; m.p. 186—190° (hydrated) and 240—242° (anhydrous), $[\alpha]_D$ −19.5° (*c* 1.25), equiv. wt. 345.5. Acidic hydrolysis gave D-glucose and D-mannose (3 : 1); partial acidic hydrolysis gave *O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose, *O*-β-D-glucopyranosyl-(1→6)-D-glucopyranose, *O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-D-glucopyranose, and an oligosaccharide (u). A periodate oxidation study showed the consumption of 8.82 mol of periodate with the liberation of 5.18 mol of formic acid per mol (691.0 g) of (p). Hydrolysis with emulsin yielded D-glucose and D-mannose, whereas after methylation by the Kuhn procedure [23,24] hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose, and 2,3,6-tri-*O*-methyl-D-mannose (1 : 2 : 1). The oligosaccharide (u), m.p. 176—180°, $[\alpha]_D$ −11.8° (*c* 0.57), gave D-glucose and D-mannose (2 : 1) on hydrolysis. Partial acidic hydrolysis yielded D-glucose, D-mannose, 4-*O*-β-D-glucopyranosyl-D-mannopyranose, and 6-*O*-β-D-glucopyranosyl-D-glucopyranose (gentiobiose). Therefore, the oligosaccharide (u) was identified as *O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose, and so the parent oligosaccharide (p) was O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosyl*-(1→4)-D-*mannopyranose* (Found: C, 41.9; H, 6.6%; equiv. wt. 345.5. $C_{24}H_{42}O_{21}$ requires C 42.1; H, 6.45%; equiv. wt. 342).

Fraction 10: *oligosaccharide* (m). The sugar (75 mg), R_{Man} 0.12 in solvent (A), R_{Glc} 0.38, 0.20, 0.14, and 0.83 in solvents (B)(i), (C)(i), (D), and (F), respectively, $R_{cellobiose}$ 0.63 in solvent (C)(i), was crystallised from aqueous ethanol; m.p. 206°, $[\alpha]_D$ +22.3° (*c* 0.50), equiv. wt. 257.8. Acidic hydrolysis gave D-glucose only. The oligosaccharide (m) was shown to belong to the series containing β-(1→4)-linked D-glucose units by the fact that its log $[R_{Glc}/(1 - R_{Glc}]$ values [R_{Glc} in solvents (B)(i), (C)(ii), (D), and (F)] lay on the appropriate lines in Figure 1 (DP 3). Micromethylation by the Kuhn procedure [23,24] gave 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-glucose (1 : 2) whereas partial acidic hydrolysis yielded D-glucose and 4-*O*-β-D-glucopyranosyl-D-glucopyranose (cellobiose), as confirmed by co-chromatography with authentic samples.

Fraction 11: *oligosaccharide* (n). The sugar (350 mg), R_{Man} 0.08 in solvent (A), R_{Glc} 0.34 and 0.22 in solvents (B)(i) and (B)(ii), respectively, $R_{cellobiose}$ 0.52 in solvent (C)(i), was crystallised from aqueous ethanol; m.p. 167—169° (monohydrate) and 210—212° (anhydrous), $[\alpha]_D$ −18.3° (*c* 2.40), equiv. wt. 273.5. Acidic hydrolysis gave mannose only, but partial acidic hydrolysis yielded *O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose and D-mannose. Micromethylation yielded 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose (1 : 2). Thus oligosaccharide (n) was identified as *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose.

Fraction 12: *oligosaccharide* (o). The sugar (630 mg), R_{Man} 0.06 in solvent (A), R_{Glc} 0.33 in solvent (B)(i), was crystallised from aqueous ethanol; m.p. 225—227°, $[\alpha]_D$ +97° (*c* 1.60), equiv. wt. 263.1. Acidic hydrolysis gave D-galactose and D-mannose (1 : 2), partial acidic hydrolysis yielded, besides galactose and mannose, *O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose and 6-*O*-α-D-galactopyranosyl-D-mannopyranose(epimelibiose). Micromethylation yielded 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-D-mannose, and 2,3,6-tri-*O*-methyl-D-mannose (1 : 1 : 1).

Fraction Y_1: *oligosaccharide* (q). The sugar (80 mg), R_{Glc} 0.22, 0.10, 0.07, and 0.68 in solvents (B)(i), (C)(ii), (D), and (F), respectively, was crystallised from aqueous ethanol; m.p. 251—252°, $[\alpha]_D$ +17.2° (*c* 0.80), equiv. wt. 313.2. Acidic hydrolysis yielded D-glucose only, whereas partial acidic hydrolysis gave glucose, 4-*O*-β-D-glucopyranosyl-D-glucopyranose(cellobiose), and *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose(cellotriose). The DP of the sugar (q) was found to be 4 (Figure 1). Micromethylation yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-glucose (1 : 3).

Fraction Y_2: *oligosaccharide* (r). The sugar (95 mg), R_{Glc} 0.10, 0.07, 0.015, and 0.48 in solvents (B)(i), (C)(ii), (D), and (F), respectively, was crystallised from aqueous ethanol; m.p. 265°, $[\alpha]_D$ +11.2° (*c* 0.79), equiv. wt. 422.2. Acidic hydrolysis gave D-glucose only, but partial acidic hydrolysis yielded glucose, 4-*O*-β-D-glucopyranosyl-D-glucopyranose (cellobiose), *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose (cellotriose), and *O*-β-D-glucopyranosyl-(1→[4-*O*-β-D-glucopyranosyl]$_2$→4)-D-glucopyranose(cellotetraose). Graphical examination showed its DP to be 5 (Figure 1).

Fraction Y_3: *oligosaccharide* (s). The sugar (110 mg), R_{Glc} 0.04, 0.05, 0.003, and 0.24 in solvents (B)(i), (C)(ii), (D), and (F), respectively, was crystallised from aqueous ethanol; m.p. 274—275°, $[\alpha]_D$ +8.4° (*c* 0.90), equiv. wt. 505.3, $R_{cellobiose}$ 0.26 and 0.28 in solvents (C)(ii) and (F), respectively, $R_{cellotetraose}$ 0.52, 0.05, and 0.37 in solvents (C)(ii), (D), and (F), respectively. It formed a β-acetate, m.p. 238—240°, and acidic hydrolysis produced D-glucose only. Micromethylation yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-glucose (1 : 5). Partial acidic hydrolysis gave glucose, cellobiose, cellotriose, cellotetraose, and cellopentaose; some unhydrolysed sugar was also detected. Graphical examination gave its DP as 6 (Figure 1).

Fraction Y_4: *oligosaccharide* (t). The sugar (295 mg), R_{Glc} 0.08 in solvent (B)(i), $R_{mannotriose}$ 0.22 [mannotriose = *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose], and $R_{cellobiose}$ 0.14 in solvent (A), was crystallised from aqueous ethanol; m.p. 216—220° (monohydrate), $[\alpha]_D$ −7.1° (*c* 1.80), equiv. wt. 504.4. Acidic hydrolysis gave D-glucose and D-mannose (5 : 1); hydrolysis with emulsin produced the same two sugars. A periodate oxidation study showed the consumption of 8.76 mol of periodate with liberation of 3.07 mol of formic acid per mol (1008.8 g) of the sugar (t). Micromethylation by the Kuhn method [23,24] yielded 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-glucose in the molecular proportions of 1 : 5. Partial acidic hydrolysis of (t) gave as many as eight smaller fractions (Z_1 to Z_8) besides D-glucose and D-mannose. The fractions Z_1 to Z_7 were single entities

but Z_8 was separated into Z_{8A} and Z_{8B}. These sugars were identified by comparing their mobilities with those already identified in solvents (B)(i) and (C)(i) (Table 4).

TABLE 4

Sugars obtained from partial acidic hydrolysis of the oligosaccharide (t)

Fraction	Sugar identified	R_{Glc} in (B) (i)	$R_{cellobiose}$ in (C) (i)
Z_1	4-*O*-β-D-Glucopyranosyl-D-glucopyranose	0.61	1.00
Z_2	4-*O*-β-D-Mannopyranosyl-D-glucopyranose	0.48	0.78
Z_3	*O*-β-D-Glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose	0.40	0.64
Z_4	*O*-β-D-Mannopyranosyl(1→4)-*O*-β-D-glucopyranosyl(1→4)-D-glucopyranose	0.37	0.42
Z_5	*O*-β-D-Glucopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_2$→4)-D-glucopyranose	0.24	0.35
Z_6	*O*-β-D-Glucopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_3$→4)-D-glucopyranose	0.11	0.18
Z_7	*O*-β-D-Mannopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_2$→4)-D-glucopyranose	0.23	0.10
Z_{8A}	*O*-β-D-Mannopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_3$→4)-D-glucopyranose	0.14	0.02
Z_{8B}	Unhydrolysed hexasaccharide	0.08	0.00

Only fraction Z_{8A} could be obtained in fairly good yield (48 mg), $[\alpha]_D$ −3.8° (*c* 0.80), but could not be crystallised; equiv. wt. 423.7. Hydrolysis of the fractions Z_7 and Z_{8A} gave D-mannose and D-glucose (1 : 3 and 1 : 4, respectively); DP values were found to be 4 and 5 by plotting log [R_{Glc}/(1 − R_{Glc})] values [R_{Glc} in solvent (B)(i)] along with that of *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose against DP (Figure 2).

Thus oligosaccharide (t) has (*a*) no branching, (*b*) β-(1→4)-linkages, and (*c*) mannose as the terminal non-reducing and glucose as the reducing group. On the basis of these facts the hexasaccharide was identified as O-β-D-*mannopyranosyl*-(1→[4-O-β-D-*glucopyranosyl*-1]$_4$→4)-D-*glucopyranose*. A plot of $[M]_D$ values of (t), *O*-β-D-mannopyranosyl-(1→[4-*O*-β-D-glucopyranosyl-1]$_2$→4)-D-glucopyranose, and *O*-β-D-mannopyranosyl(1→[4-*O*-β-D-glucopyranosyl-1]$_3$→4)-D-glucopyranose against DP gave a straight line (Figure 2), supporting the assigned structure having DP 6 (Found: C, 42.65; H, 6.5. $C_{36}H_{62}O_{31},H_2O$ requires C, 42.85; H, 6.35%).

Determination of R_{Glc} *Values of Higher Cellodextrins.*—Spots of penta- and hexa-saccharides along with a reference spot of cellotetraose were placed on a sheet (4 × 22 in) of Whatman No. 1 paper. The paper was irrigated continuously with solvent (D) for 3 days and the spots were located as usual. The expression $R_{Glc} = 0.07a/b$ was applied, where *a* and *b* are the distances of the spot and the cellotetraose, respectively, from the base line (0.07 is R_{Glc} of cellotetraose under similar conditions).

Separation of Monosaccharides.—The solution collected from over-running of the chromatograms used for separating oligosaccharides on Whatman No. 3 MM sheets, was distilled down to *ca.* 50 ml. A sample (10 ml) was resolved chromatographically into four fractions (M_1 to M_4). The sugars were identified by co-chromatography and further characterized by their physical constants (Table 5).

TABLE 5

Yields and physical constants of monosaccharides obtained from partial acidic hydrolysis of the polysaccharide from *C. tora* seeds

Fraction no.	Yield in 10 ml (mg)	Yield in Total hydrolysate (g)	Cryst. from	M.p. and mixed m.p. (°C)	$[\alpha]_D$ (°)	Sugar identified
M_1	160	0.80	H_2O–MeOH	151	+25	D-Xyl
M_2	550	2.75	H_2O–EtOH	132	+12.6	D-Man
M_3	74	0.37	H_2O–EtOH	146	+56	D-Glc
M_4	216	1.08	H_2O–MeOH	162—163	+80	D-Gal

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