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Polysaccharides from thermal polymerization of glucosides

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ABSTRACT

As a first step towards synthesis of labeled polysaccharides for studies of mechanisms of pyrolysis, the acid-catalyzed thermal polymerizations of methyl α-D- and phenyl β-D-glucopyranosides have been studied and the optimum yields of polymer determined. The phenyl aglycone is a more facile leaving group than methyl and hence the phenyl glucoside yields polymer more rapidly at lower temperature. However, the byproduct phenol is less rapidly removed than methanol during polymerization, and the polysaccharide products from the phenyl glucoside are less pure. The polymer from methyl α-D-glucopyranoside is a mixed glucopyranan of average degree of polymerization (d.p.) 10–13, as determined by three different methods. The end groups are methyl α-D-glycopyranoside units, the predominant in-chain linkage, determined by methylation analysis, is 1→6, and the predominant branch points are 3,6-linked. Both α- and β-glucopyranosidic linkages are present in-chain in the approximate ratio 2:1. The similar, acid-catalyzed thermal polymerization of 1,6-anhydro-β-D-glucopyranose (levoglucosan, LG) has also been studied, and the mechanisms of the polymerizations are discussed.

INTRODUCTION

In the course of a systematic study of the mechanisms of thermal degradation of polysaccharides, we have shown that generally 1,6-anhydro-β-D-glucopyranose (levoglucosan, LG) and glycolaldehyde (GA) are the two major volatile products from glucans, and that their formation occurs by competing pathways[1]. LG formation is favored by the removal of all ionic material, especially metal ions, before pyrolysis. On the other hand, the yield of GA is increased at the expense of LG by the presence or addition of very small amounts of "neutral" salts such as sodium chloride. We have postulated a mechanism of LG formation from glucans which is dependent on the stability and volatility of LG in "escaping" from a system of equilibria in the pyrolyzing solid[1], and have obtained some support for this hypothesis by a study of the pyrolysis of a model compound (cellobiitol)[2]. The mechanism of GA formation in the pyrolysis of polysaccharides is not known, although some speculations have been made[3,4]. In an attempt to investigate this question we seek to prepare some model hexosans in which there are appropriate isotopic labels at C-1 and C-6, respectively. The presence or absence of the label in GA produced by pyrolysis of the hexosan may indicate likely mechanisms for GA formation. It appeared to us that our aim would be best achieved by the synthesis of labeled glycosides, which could then be thermally polymerized to

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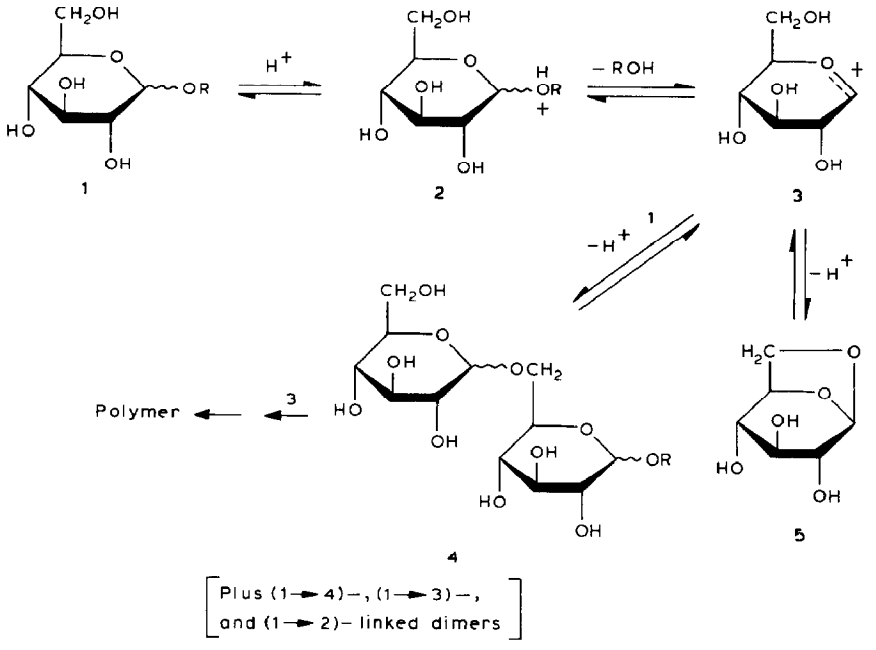
polysaccharides for subsequent pyrolysis experiments. Thus we have commenced an investigation of the thermal polymerization of some glucosides. In the course of this study, it became evident that the possible involvement of LG as an intermediate in glucoside pyrolysis must be considered. Hence we have found it necessary to reinvestigate the thermal polymerization of LG, despite the extensive earlier literature on this reaction.

Wollwage and Seib[5] have proposed a mechanism for the acid-catalyzed polymerization of LG that involves rate-controlling formation of a carbonium-oxonium ion intermediate by C-1–O-6 scission. In the subsequent addition of oxygen nucleophiles to this reactive intermediate, little selectivity would be anticipated for the formation of either α or β linkages, and structural studies had previously shown that both types of linkages are present in the polymer[6,7]. Among the driving forces for the scission of the anhydride ring to the glucosyl cation are the resonance delocalization of the positive charge and the relaxation of the strained conformation of the anhydro sugar[8]. Further evidence for the formation of a resonance-stabilized glucosyl cation from LG was subsequently presented in a study on the kinetics and mechanism of the acid-catalyzed butanolysis of LG by Kieboom and co-workers[9].

The thermal polymerization of glycosides was first observed with 2-deoxyfuranosides by Deriaz *et al.*[10] and by Stacey *et al.*[11]. The latter workers found that the reaction was a condensation polymerization which, in the case of 2-deoxy-α,β-D-galactofuranoside[12], resulted in a predominantly (1→6)-linked linear polymer of 4–8 furanose units (cryoscopic). The thermal polymerization of glycopyranosides was first observed by Liskowitz and Carroll[13] with the methyl α-glycosides of D-glucose and D-mannose. Shafizadeh and co-workers[14,15] observed Lewis-acid catalysis in the thermal polymerization of β-D-xylopyranosides. The latter workers noted similar behavior by aryl β-D-glucopyranosides[16] and found that the corresponding 2-deoxyglycosides polymerized more readily and at lower temperatures. McGinnis and Parikh[17] later showed that condensation polymerization is the major reaction occuring early in the thermal decomposition of methyl α-D-glycopyranosides. Analysis by Lai and Shafizadeh[18] of the polymeric material formed in the zinc chloride-catalyzed thermolysis of phenyl β-D-glucopyranoside suggested that it consisted of randomly linked and branched polymers of glucopyranose.

The mechanism of thermal polymerization of glycosides (as for LG) can be postulated as shown in Scheme 1. A mass of experimental evidence supports the conclusion that the glucosyl cation **3** is also an intermediate in the analogous acid-catalyzed hydrolysis of glucopyranosides, and that it forms by rapid and reversible protonation of the aglyconic oxygen followed by unimolecular heterolysis of the carbon to oxygen bond[19]. The analogy is consolidated by observations that 2-deoxypyranosides, which are especially susceptible to acid hydrolysis, are particularly prone to thermal polymerization[10–12]. In addition, work by Capon and Thacker[20] has shown that such a glucosyl cation is an intermediate in the solvolysis of methyl β-D-glucopyranoside.

In the present study we have investigated the acid-catalyzed polymerization of



Scheme 1

LG and of methyl α-D- and phenyl β-D-glucopyranosides and have investigated the molecular weights and structures of the polymers formed. All our observations are compatible with the mechanism shown in Scheme 1.

RESULTS AND DISCUSSION

A polymer was obtained from LG by heating with monochloroacetic acid. Indications of the approximate weight-average molecular weight of the LG polymer were obtained by two chromatographic methods. On h.p.l.c., the polymer eluted at a rate similar to that of maltopentaose. Gel-permeation chromatography on Sephadex G-50 (Fig. 1) indicated $d.p._w \sim 7$ on the basis of calibration values provided by the gel manufacturer and based on dextran reference compounds[21]. This result was supported by similar chromatography on Sephadex G-25 (Fig. 2). Neither determination of the $d.p._w$ of the polymer should be regarded as absolute in view of the major chemical differences between the LG polymer and the reference glucans.

Thermal polymers from LG have been shown by previous workers to contain LG end groups. Thus, Wolfrom *et al.*[6] detected LG after partial hydrolysis of their LG polymer, and subsequently showed[22] that the dimeric products of their polymerization contained LG end groups and hence lacked (1→6)-links.

The results of a methylation analysis of the LG polymer produced in this study are shown in the last column of Table IV. The figures give the abundance of each glucitol derivative relative to the 2,3,4,6-tetra-*O*-methyl compound. In assessing these results we

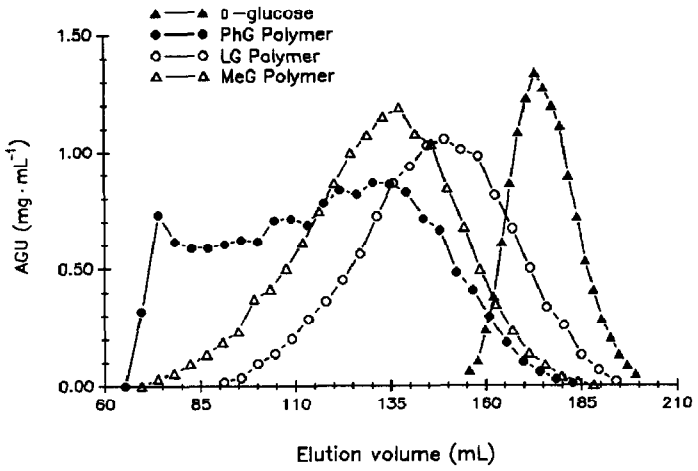


Fig. 1. Gel-permeation chromatography on Sephadex G-50. AGU = anhydroglucose units.

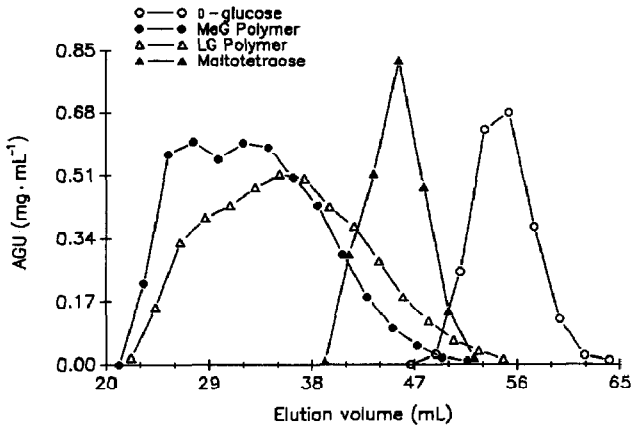


Fig. 2. Gel-permeation chromatography on Sephadex G-25. AGU = anhydroglucose units.

assume that all end groups in the LG polymer are LG and that d.p. is about 6. The LG groups could only yield dimethyl or monomethyl derivatives and the sums of these from Table IV are respectively 0.89 and 0.30, *i.e.*, together they are approximately equal to the relative value of 1.09 for the "other" end-group products, *viz.* the tetramethyl ethers. The "in-chain" linkage positions are distributed among the available four hydroxyl groups with some preference for the 6-position. All of the preceding conclusions are compatible with the mechanism shown in Scheme 1, with cation **3** being generated from protonated LG. The formation of "in-chain" units occurs by the addition of **3** (or at later stages in the reaction, higher homologous cations) to an intermediate oligomer. It is evident from the distribution of trimethyl derivatives in Table IV that this addition occurs most readily at a C-6 hydroxyl. This selectivity is evidently controlled by steric factors and is analogous to the known preferential addition of the fructofuranosyl cation to primary alcohols in comparison with secondary alcohols[22].

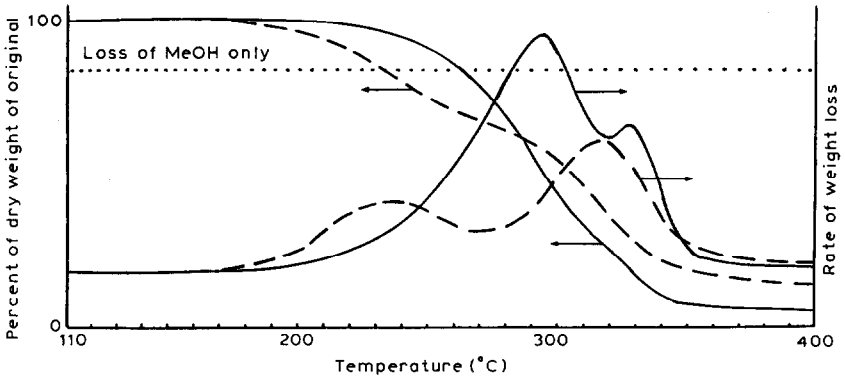


Fig. 3. Thermal analysis of methyl α-D-glucopyranoside. ——, neat; ---, with 1% trichloroacetic acid. Arrows indicate applicable axes.

Previous workers have also shown that acid catalyzes the polymerizations of both LG (ref. 7) and glucosides[14,15,18]. In the present work, thermogravimetric analyses of methyl and phenyl glucosides show clearly that trichloroacetic acid (TCA) catalyzes the formation of polymer as the sample is heated (we chose this acid rather than the monochloroacetic acid used by most previous workers because of its greater acidity and higher boiling point). Figure 3 shows thermogravimetric (t.g.) curves and the corresponding differential thermogravimetric (d.t.g.) curves for the heating of methyl α-D-glucopyranoside with and without the addition of 1% TCA. The horizontal line near the top of the figure corresponds to the theoretical weight loss resulting from the scission of all glycosidic bonds with the escape of methanol. The two maxima in each d.t.g. curve correspond to two major thermal events, which we interpret respectively as loss of the aglycone to form the polymer and decomposition of the polymer. Without acid catalysis, the first of these maxima occurs at a temperature which on the t.g. curve corresponds to a weight loss of about three times the theoretical for the loss of methanol, indicating that elimination (*i.e.*, char forming) reactions are occuring simultaneously with polymer formation. The addition of 1% acid lowers the temperature at which the first thermal event occurs, and the d.t.g. maximum for that event matches closely the reduction in weight corresponding to the loss of methanol. Moreover, the addition of 1% acid, while evidently catalyzing polymer formation, has less effect on the subsequent decomposition of the polymer. It was found that the addition of larger concentrations of acid catalyze both the formation and the decomposition of polymer.

Some representative polymerizations conducted in this study are listed in Table I. Yields are given in percent of theoretical based on starting material. Within the range of conditions used, increasing acid concentration resulted in increased yield of polymer from the methyl glucoside. The last column gives each polymer's apparent glucan content as determined by phenol–sulfuric analysis[24]. The lower apparent glucan contents for the polymers from the phenyl glucoside are partly accounted for by the larger aglycone (present on the end group), but there are probably also small amounts of noncarbohydrate components, possibly dehydration products[18], in all of the polymers.

TABLE I

Glucans from the thermal polymerization of glucosides

Run no.	*Substrate, catalyst*	*Polymerization conditions*	*Isolated yield of polymer[a] (%)*	*Apparent anhydroglucose content[b] (%)*
1	α-MeG[c] (neat)	220°, 2.5 h	9	92
2	α-MeG, 0.1% TCA[d]	210°, 2.5 h	25	89
3	α-MeG, 1% TCA	175°, 2.5 h	53	92
4	α-MeG, 5% TCA	158°, 3.5 h	57	90
5	β-PhG[e] (neat)	240°, 3 h	36	82
6	β-PhG, 1% TCA	190°, 3 h	48	86
7	β-PhG, 1% TCA	200°, 2 h	66	79

[a] Fraction insoluble in 95% ethanol. [b] By phenol–sulfuric acid analysis. [c] Methyl α-D-glucopyranoside. [d] Trichloroacetic acid. [e] Phenyl β-D-glucopyranoside.

TABLE II

Thermal polymerization of methyl α-D-glucopyranoside

Run no.[a]	*Weight loss[b] (%)*	*Components of ethanol-soluble fraction*		
		Oligosaccharide (%)	*LG (%)*	*MeG (α + β) (%)*
1	15.4	67	1.9	18
2	16.2	56	5.7	11
3	16.7	38	1.3	5.4
4	15.2	19	3.3	15

[a] Correspond to run numbers in Table I. [b] Theoretical for complete loss of methanol, 16.5%.

TABLE III

Thermal polymerization of phenyl β-D-glucopyranoside

Run no.[a]	*Weight loss[b] (%)*	*Components of ethanol-soluble fraction*			
		Oligosaccharide (%)	*Phenol (%)*	*LG (%)*	*β-PhG (%)*
5	37.6	50	6.8	4.1	1.1
6	31.5	54	3.8	0.5	0.4
7	37.2	25	11.2	1.3	0.2

[a] Correspond to run numbers in Table I. [b] Theoretical for complete loss of phenol, 36.7%.

In accordance with this proposal, it is noteworthy that the methyl glucoside polymers were white, whereas the phenyl glucoside polymers were brown in color, with the last polymer listed in Table I showing the most color.

Tables II and III show the weight loss achieved during the polymerizations, and

TABLE IV

Methylation analysis of PhG, MeG, and LG polymers by g.l.c.-m.s. of the acetylated *O*-methyl glucitols

Glucitol (methylated positions)	*Relative retention time*[a]	*Relative molar ratio*		
		PhG polymer	*MeG polymer*	*LG polymer*
2,3,4,6	**1.00**	**1.00**	**1.00**	**1.00**
2,3,5,6	1.09	0.10	0.18	0.09
2,4,6	1.46	0.30	0.31	0.28
3,4,6	1.50	0.44	0.47	0.42
2,3,4	1.72	0.83	0.77	0.67
2,3,6	1.76	0.45	0.47	0.51
2,4	2.79	0.40	0.40	0.34
2,3	2.97	0.32	0.30	0.28
3,4	3.00	0.32	0.29	0.27
2	4.04	0.09	0.07	0.10
3	4.50	0.08	0.06	0.11
4	5.11	0.07	0.06	0.09

[a] On an SP2330 fused silica capillary column.

the yields of the components of the ethanol-soluble fractions (see Experimental). The oligosaccharides of the ethanol-soluble fraction were shown by t.l.c. analysis to range in size up to about d.p. 4. In the same fraction, g.l.c. of trimethylsilylated samples showed both anomeric methyl glucopyranosides, with the β anomer amounting to 10% of the quantity of α-anomer in the acid-catalyzed runs. The β anomer results from recombination of the cation **3** with methanol before the latter is lost from the melt. The relatively small amount of unchanged starting material shown in Table IV is explained by the fact that the phenoxy group is a better leaving group than the methoxy group. The presence of LG in the ethanol-soluble fractions is accounted for by the reversible side reaction shown in Scheme 1.

The question of whether or not LG might serve as an intermediate in the polymerization of glucosides motivated an experiment designed to measure the relative rates of polymerization of LG and the methyl glucoside. The results indicated that LG polymerizes five times faster than methyl α-D-glucopyranoside, with first-order rate constants $k_{LG} = 0.10 \pm 0.02\ s^{-1}$ and $k_{MeG} = 0.019 \pm 0.001\ s^{-1}$. The rate determining step for the polymerization of both LG and glycosides is almost certainly the formation of **3**. Hence, if the polymerization pathway for the glucoside (**1**) passed through LG (**5**), the faster polymerization of **5** should militate against the presence of significant LG in the melt. Since substantial proportions of LG were sometimes found (see runs 2, 4, and 5, Tables II and III), we tentatively conclude that the glucoside polymerization does not proceed *via* LG. It should be noted in passing that Wollwage and Seib[5], measuring the rate of acid-catalyzed thermal polymerization of LG, observed a lag phase, during which the monomer disappeared very slowly, preceding the first-order phase of the reaction. This was due to the use of a reaction temperature (115°) well below the melting point of LG (179–180°) and to the heterogeneity of the reaction mixture, the acid

catalyst being applied as a coating on the LG crystals. In our procedure the lag phase was eliminated by more intimately mixing the acid with the LG (see Experimental) and by carrying out the reaction at a higher temperature (170°).

Hydrolysis of polymers from run no. 3 (MeG polymer) and from run no. 6 (PhG polymer), followed by determination of the released methanol and phenol, respectively, provided confirmation of the presence of glucosidic end groups as predicted by Scheme 1. Further evidence for such end groups was furnished by a ^{1}H-n.m.r. spectrum of the MeG polymer showing a strong methoxy singlet. The hydrolyses also provided the means for determining the values of $d.p._n$, by measurement of methanol and phenol yields, respectively, and assuming one aglyconic end group per glucan molecule. These results indicated a $d.p._n$ of 13 for the MeG polymer and of 16 for the PhG polymer.

Gel-permeation chromatography (Fig. 1) was used to determine approximate values for $d.p._w$, subject to the reservations mentioned above on the relevance of reference compounds. The results show that the PhG polymer ($d.p._w \sim 20$) was larger, and the gel chromatogram suggests a larger spread of molecular weights than for the MeG polymer ($d.p._w \sim 12$). The ratio of in-chain to end-group anomeric proton signals in the n.m.r. spectrum of the MeG polymer provided an additional determination for this polymer and indicated $d.p._n \sim 10$. These molecular weight values are rather lower than those for glucans which have been made by acid-catalyzed thermal polymerization of glucose. Such polymers, produced by Mora and co-workers[25], have apparent d.p.'s well in excess of 100, as determined by reducing end-group methods[25,26]. It should be noted, however, that if any of the "reducing" end groups are LG groups, then such methods would yield falsely high d.p. values, and in fact d.p. values measured by osmotic and viscosity methods were 42 and 37 respectively.

As with the LG polymer, additional information about the structures of the MeG polymer and the PhG polymer was obtained by methylation analysis. The results (Table IV) indicate similar structures, and thus similar mechanisms of formation, for all of the polymers. The monomethyl derivatives from the glucoside-derived polymers correspond to double branch points, and their presence, together with substantial portions of dimethyl derivatives, indicates a high degree of relatively random branching. As with the LG polymer, the most abundant trimethyl derivative is the 2,3,4 isomer, derived from an anhydroglucose unit in the polymer having its sterically accessible 6 hydroxyl substituted. Likewise, the most abundant dimethyl derivative is the 2,4 isomer, derived from a monomer unit with optimum spacing for three linkages on a glucopyranose ring (*i.e.*, 3,6-disubstitution). Table V lists the percentage of different types of linkages present in each polymer. The roughly equal amounts of (1→4)- and (1→2)-links, in both the LG and the glucoside polymers, confirms that the polymerization mechanism does not involve the ionization of hydroxyls, since it is known that the 2-hydroxyl group is the most acidic in an unsubstituted methyl glucoside[27].

The 2,3,5,6-tetra-*O*-methyl derivatives listed in Table IV derive from terminal furanoside units. These constitute 8–15% of those terminal units linked *via* C-1, and their presence raises the question of mechanism whereby furanose rings could arise from the glucosyl cation **3**. Such a mechanism has been proposed by Pater, Coelho, and

TABLE V

Linkages present in the polymers

Polymer	*Linkages (% of total)*			
	1→6	*1→4*	*1→3*	*1→2*
PhG	44	19	18	19
MeG	43	20	18	19
LG	40	22	18	20

Mowery[28]. Goldstein and Hullar[29] have proposed a mechanism for the polymerization of free sugars which involves the cation **3** as intermediate, and furanose end groups have been detected in glucans made by acid-catalyzed thermal polymerization of glucose[25]. A methylation analysis of one such glucan conducted by Dutton and Unrau[26] showed that 20% of the tetramethyl derivatives were the 2,3,5,6 isomer. In addition to furanose end groups, it is quite probable that our glucans have furanose rings incorporated within the chain, although we were not able to unequivocally identify the derivatives stemming from such units.

Analysis by ^{1}H-n.m.r. indicated that there were about twice as many α linkages as β linkages in the MeG polymer. This ratio is similar to that obtained in the reaction of glucose with methanol and an acid catalyst, which results in a preponderance of the α-glucopyranoside[30,31].

EXPERIMENTAL

Thermogravimetric analyses. — The TG system was based on a Cahn R-100 electrobalance, with a resistively coated, temperature-programmed furnace surrounding the sample pan. Temperature was measured by a thermocouple positioned 1 mm below the pan. The assembly was interfaced to a laboratory microcomputer system for data acquisition and for control of the temperature program. This system consisted of a Tektronix 4051 microcomputer plus a ROM-based A/D converter and a real-time clock (Trans-Era), and a Hewlett–Packard Model 6002A programmable power supply for the furnace. Samples sizes ranged from 10 to 15 mg. Acid-treated samples were prepared as described below. During heating, the sample was maintained under nitrogen flowing at 30 mL.min^{-1}. The temperature program was set to start at 50°, heat to 110° at 20°.min^{-1}, hold at 110° for 10 min, and then heat to 400° at 10°.min^{-1}. Differential thermal gravimetric curves were plotted by a Tectronix 4662 interactive digital plotter interfaced with the computer.

Thermal polymerization of LG. — LG (0.37 g) and monochloroacetic acid (4.4 mg) were dissolved in MeOH (1.0 mL) and water (0.15 mL). The solution was taken to dryness under a stream of filtered air at room temperature to form a glass. The test tube was evacuated to 1 torr, sealed, and placed in an oil bath for 30 min at 170°. The melt was redissolved in water (2 mL) and added dropwise with stirring to 40 mL of absolute

ethanol to precipitate the polymer, which was subsequently washed by centrifugation twice with 95% ethanol and twice with acetone, then dried to white powder at 40°/1 torr.

Thermal polymerizations of glucosides. — Acid-treated glucoside samples were prepared by mixing trichloroacetic acid (TCA) with methyl α- or phenyl β-D-glucopyranoside in water or ethanol solution, respectively. These solutions were then taken to dryness in a vacuum oven at 40° to form glasses. Polymerization was effected by isothermal heating of 1 g samples in open, flat-bottomed vials in a thermostated oven (± 1°). During heating, the vials were removed at 30 min intervals and weighed, so that the heating could be stopped when the weight loss was near theoretical. The final melt was dissolved in water (5 mL) and added dropwise with stirring to absolute ethanol (100 mL) to precipitate the polymer, which was subsequently washed and dried as just described.

G.l.c. analysis of ethanol-soluble fractions. — After precipitation of the polymer, the supernatant solution was evaporated to dryness and a sample was derivatized for g.l.c. analysis with 1:1 (v/v) bis(trimethylsilyl)trifluoroacetamide in pyridine, after the addition of D-glucitol as internal standard. The resulting trimethylsilyl (TMS) ethers were analyzed on a nickel column (2.2 mm o.d. × 2.4 m) packed with 3% of SE-52 on GasChrom Q (100–120 mesh). The temperature was programmed to increase from 130° to 250° at $6°.min^{-1}$. The g.l.c. analyses featured nitrogen as the carrier gas, flame-ionization detection, and digital integration.

T.l.c. analysis of MeG oligosaccharides. — The ethanol-soluble fraction from polymerization no. 3 was examined by t.l.c. on Baker-flex Silica Gel IB2 using 3:1:1 methyl ethyl ketone–acetic acid–water as solvent. Molecular weight estimates were made by reference to maltose oligosaccharides.

Determination of rate constants. — Methyl α-D-glucopyranoside (0.36 g) was dissolved with heating in 95% ethanol (5 mL). The solution was cooled, aqueous TCA (3.6 mg of acid) was added, and the mixture was then dried to a glass under a stream of filtered air. A mixture of LG (0.31 g) with TCA (3.1 mg) was similarly prepared. These samples were then heated in open test tubes in an oil bath at 170 ± 0.1°. Small quantities (10–20 mg) of the melts were removed at 10 min intervals, D-glucitol was added, and the samples were analyzed by g.l.c. as described above to determine the remaining starting material.

Hydrolysis of end groups from glucoside polymers. — Methyl α-D-glucopyranoside (5 mg) and the MeG polymer (10 mg) were dissolved in 0.5M sulfuric acid (0.1 mL) in separate small test tubes. The test tubes were sealed and placed in an oil bath for 2 h at 110°. The contents were then neutralized with barium carbonate and analyzed for methanol by g.l.c., using a Chromosorb 102 80/100 column at 90° isothermal in conjunction with flame-ionization detection and digital integration.

A similar procedure was applied to phenyl β-D-glucopyranoside and the PhG polymer. Phenol determination was achieved using a Perkin–Elmer Lambda 3B UV/VIS spectrophotometer (λ_{max} 287 nm, ε = 2760 nm for phenol after the addition of sodium hydroxide to 1M).

N.m.r. analysis of MeG polymer. — The MeG polymer was dissolved in D_2O (5

mL), freeze dried, and then redissolved in D_2O (1 mL) containing 2-methyl-2-propanol as internal standard (δ 1.20) for recording of the ^{1}H-n.m.r. spectrum. The spectrum was very complex, and only the *O*-methyl and anomeric proton signals were assigned, as follows: OCH_3,δ 3.36; in-chain H-1α (*eq*), 4.95; in-chain H-1β (*ax*), 4.48; and reducing-end H-1α (*eq*), 4.60 (ref. 31). The first two anomeric-proton signals appeared as broad singlets, while the third was sharper, but still not resolved into a characteristic doublet. Integration of these three signals showed that the sum of the areas of the first two was 9.7 times the area of the third, suggesting a d.p. value of 10.

H.p.l.c. analysis of LG polymer. — An aqueous solution of the LG polymer was injected onto a Waters Associates C_{18} Radial-PAK column (100 × 8 mm i.d., 10μm), which was eluted with distilled water at a flow rate of 4 mL.min^{-1}. The chromatogram was compared to that of a maltose oligosaccharide solution injected under the same conditions. The major peak of the levoglucosan polymer coincided with the peak for maltopentaose.

Gel-permeation chromatography. — Samples (20–30 mg) of the MeG polymer, the PhG polymer, and the LG polymer were separately eluted from a column (26 mm i.d. × 320 mm) of Sephadex G-50 (Pharmacia Biotechnology Products) with distilled water. The void volume was determined with Blue Dextran 2000 (Pharmacia Fine Chemicals), and the permeation limit with glucose. Data for elution curves were obtained by phenol–sulfuric acid analyses[24]. The curves are shown in Fig. 1, and similar profiles from a G-25 column (16 mm i.d. × 320 mm) in Fig. 2.

Methylation analysis. — Samples (10 mg each) of the three polymers were methylated by a treatment similar to the Hakomori procedure but differing in that finely powdered sodium hydroxide was used as the base rather than sodium hydride. The effectiveness of this method, previously demonstrated[33,34], was confirmed by the complete absence of hydroxyl absorption in the F.t.i.r. spectrum of one of the methylated samples. The methylation mixtures were dialyzed overnight to isolate the polymer, which was then hydrolyzed with 90% followed by 15% trifluoroacetic acid. Reduction was achieved with 1% $NaBH_4$ in M ammonium hydroxide, and acetylation with acetic anhydride in pyridine. Partitioning between ethyl acetate and water was followed by dissolution of the partially methylated glucitol acetates in acetone, preparatory to g.l.c.–m.s. analysis.

Analysis of partially methylated alditol acetates. — G.l.c. and m.s. data were obtained using a Supelco 2330 fused silica capillary column (30 m, 0.25 mm i.d.) in a Hewlett–Packard 5890A gas chromatograph. The glucitol derivatives resulting from the methylations were analyzed quantitively by using the above column at 220° isothermal in conjunction with flame-ionization detection and digital integration. The molar ratios shown in Table IV have been corrected by the application of response factors derived from e.c.r. theory[35]. Qualitative analysis was achieved by using the column in conjunction with a Hewlett–Packard 5970 mass-selective detector interfaced with a Hewlett–Packard 9133 computer. The temperature was programmed to increase at 20°.min^{-1} from 60°, and was held at 245°; all of the derivatives eluting during the isothermal stage. Mass spectra of the derivatives were matched to those of authentic compounds[36]. Helium carrier gas was used for both the g.l.c.–m.s. and g.l.c.–f.i.d. work.

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