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A novel fructoglucan from the thermal polymerization of sucrose

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ABSTRACT

The heating of anhydrous, amorphous, acidified sucrose produces a novel fructoglucan in ~30% yield; it contains D-fructose and D-glucose in a ~1:2 ratio. It is highly branched and of relatively low molecular weight; gel-permeation chromatography indicates an average dp ~25. Methylation analysis was complicated by acid degradation of the methylated fructose units under conditions for hydrolysis of glucopyranoside linkages. Parallel application of three different, hydrolytic regimes indicated linkages predominantly through O-6 of glucopyranoside and O-1 of fructofuranoside residues. Most of the nonreducing end groups are glucopyranose and both single and double branch-points are present. The fructoglucan is similar in molecular size and architecture to a commercial glucan, Polydextrose™, which finds extensive use as a noncalorific food-bulking agent.

INTRODUCTION

Following our recent work[1] on the formation of kestoses (trisaccharides) by the thermolysis of sucrose under comparatively mild conditions, the polymerization of sucrose has been studied under more rigorous conditions. The fructosyl cation, formed by the scission of sucrose, may be expected to undergo nucleophilic attack by hydroxyl groups from any of the species in the reaction medium. Thus, reaction could occur with sucrose, glucose (also formed by the scission of sucrose), and oligosaccharides that form during the early part of the reaction. Other oligomers, containing fructofuranoside linkages internally, may also undergo scission to yield cations of the form $Glc_mFru_n^+$. These and other possible reactions are outlined in Scheme 1.

The experiments yielded a novel fructoglucan, obtained in ~30% yield after precipitation by ethanol from aqueous solution. The characterization of this

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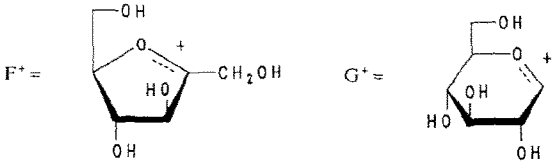
$$G\text{-}O\text{-}F \rightarrow F^{+} + G\text{-}O^{-}$$
$$F^{+} + G\text{-}O\text{-}F \rightarrow G\text{-}O\text{-}F\text{-}F \text{ or } F\text{-}G\text{-}O\text{-}F$$
$$G\text{-}O^{-} + H^{+} \rightarrow G\text{-}OH$$
$$F^{+} + G\text{-}OH \rightarrow F\text{-}G\text{-}OH$$
$$G\text{-}OH \rightarrow G^{+}$$
$$G^{+} + G\text{-}OH \rightarrow G\text{-}G\text{-}OH$$
$$F^{+} + G\text{-}OH \rightarrow F\text{-}G\text{-}OH$$
$$F^{+} + F\text{-}G\text{-}OH \rightarrow F\text{-}F\text{-}G\text{-}OH$$
$$F^{+} + G\text{-}O\text{-}F\text{-}F \rightarrow G\text{-}O\text{-}F\text{-}F\text{-}F \text{ or } F\text{-}G\text{-}O\text{-}F\text{-}F$$
$$G^{+} + G\text{-}O\text{-}F\text{-}F \rightarrow G\text{-}O\text{-}F\text{-}F\text{-}G \text{ or } G\text{-}G\text{-}O\text{-}F\text{-}F$$
$$G\text{-}O\text{-}F\text{-}F\text{-}G \rightarrow G\text{-}F\text{-}F^{+} + G\text{-}O^{-}$$
$$G\text{-}F\text{-}F^{+} + G\text{-}G\text{-}OH \rightarrow G\text{-}F\text{-}F\text{-}G\text{-}G\text{-}OH$$

etc. G–O–F = Sucrose, G–OH = Glucose, G–O–F–F, and F–G–O–F = Kestoses



Scheme 1. Possible patterns of thermal polymerization in acidified sucrose melts.

fructoglucan is described herein and an oligosaccharide fraction, formed concurrently, will be reported upon later. In view of the recent interest in use of fructooligosaccharides to induce beneficial effects on intestinal microflora[2] the various products of thermolysis of sucrose are under further investigation.

Fructoglucans do not occur in Nature, although a number of fructans and related oligosaccharides terminating in sucrose residues are found, for example, inulin, levan, and oligosaccharides of the kestose series.

RESULTS AND DISCUSSION

Anhydrous, amorphous, acidified sucrose containing 1% by weight of citric acid was polymerized by heating under a variety of conditions; the choice and nature of reactants were discussed in ref. 1. Table I outlines yields and some of the properties of the polymeric (that is, ethanol-precipitable) products of these experiments. The absorbance at 450 nm indicates the degree of visible color. These results show that increased yield of precipitable material may be obtained by heating for longer periods or at a higher temperature. However, these conditions results in increased color. Visible color was slightly inhibited when the heating process took place in a vacuum. For purposes of comparison, polymerizations were also undertaken using fructose and glucose (both with 1% citric acid) and the results of these experiments are listed in Table II. The thermal polymerization of glucose has been well documented[3], and the product of this reaction was virtually

TABLE I

Yields resulting from the polymerization of sucrose under a variety of conditions

Temp (°C)	Time of heating (h)	Conditions	A_{450} (nm)	Yield [a] (%)
125	6	air	[b]	10
125	9	air	2.1	15
125	18	air	3.3	20
125	30.5	air	4.5	32
125	46	air	5.2	38
125	46	vacuum	3.5	39
140	3	air	1.7	18
155	1	air	1.2	18
170	0.33	air	[b]	8
170	0.83	air	2.2	25
170	1.33	air	3.9	34
170	1.66	air	4.4	37

[a] Polymer precipitable in 95% ethanol. [b] Not determined.

colorless. The fructose product showed considerable color, indicating that acid degradation of fructose was occurring. The low yield of polymer from fructose indicates that formation of fructosyl cation occurs at a lower rate than from sucrose, due perhaps to the preponderance of the pyranose form. The product of heating sucrose with 1% citric acid for 80 min at 170°C in air was chosen for further study, since this represented the best compromise of yield, color, and practicality of preparation. This product is here referred to as the fructoglucan.

Gel chromatography.—Three different gels were used for size-exclusion chromatography of the fructoglucan. Fig. 1 shows the trace obtained from Bio-Gel P-6; the dotted line shows the trace from commercial Polydextrose™ (from a similar polymerization of glucose) on the same gel. Commercial literature (Pfizer Pharmaceutical) indicates that 88.7% of Polydextrose™ falls in the molecular-weight range 162–5000 (dp 1–31), based upon Sephadex chromatography. The grid indicating the relative elution positions of the maltooligosaccharides was obtained from the 1992 work of Hoffmann et al.[4], who used similar chromatography conditions. It is intended only as a rough guide to dp, since these standards were run on a different column than our samples. It is unwise to speculate in detail upon the molecular weight of the fructoglucan based upon evidence from size-ex-

TABLE II

Yields resulting from the polymerization of fructose and glucose

Components	Yield [a] (%)	Yield after dialysis (%)
Fructose	8	6
Glucose	81	22

[a] Conditions used: 30 h, 125°C, air, polymer yield as Table I.

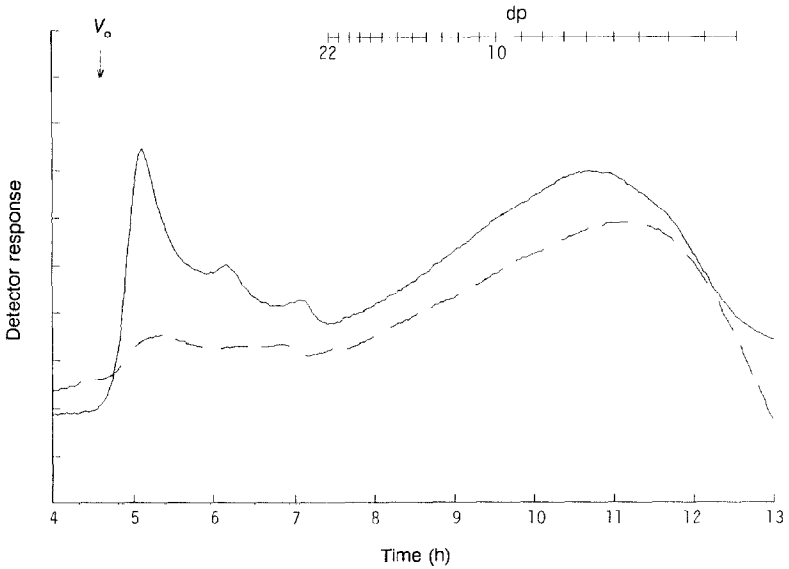


Fig. 1. Gel chromatography of fructoglucan (———) and of Polydextrose™ (— — —) on Bio-Gel P-6. Calibration with maltodextrins, RI detection.

clusion chromatography, because the highly branched nature of the fructoglucan, the presence of both fructose and glucose residues, and the wide variety of linkages revealed by methylation analysis (see later), all tend to affect the chromatographic behavior of the sample[5]. Preparative gel chromatography was carried out on Sephadex G-100 (Fig. 2). Roman numerals indicate the fractions into which

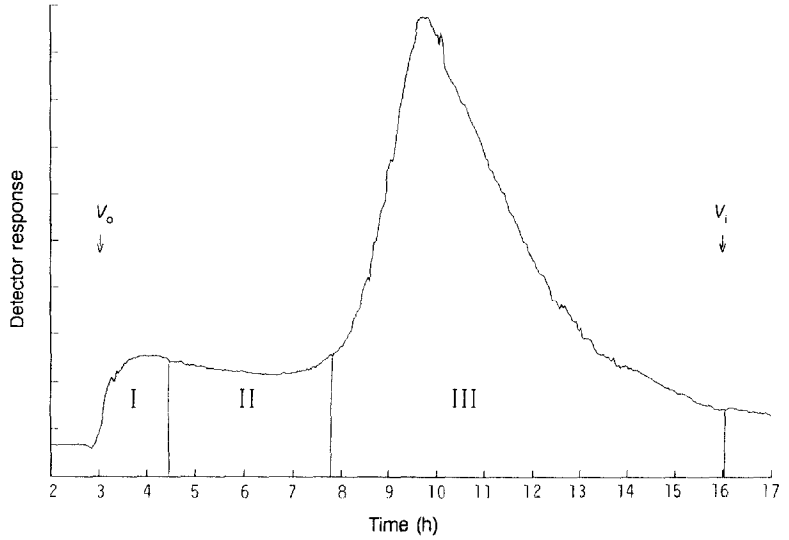


Fig. 2. Gel chromatography fractionation of fructoglucan on Sephadex G-100, RI detection.

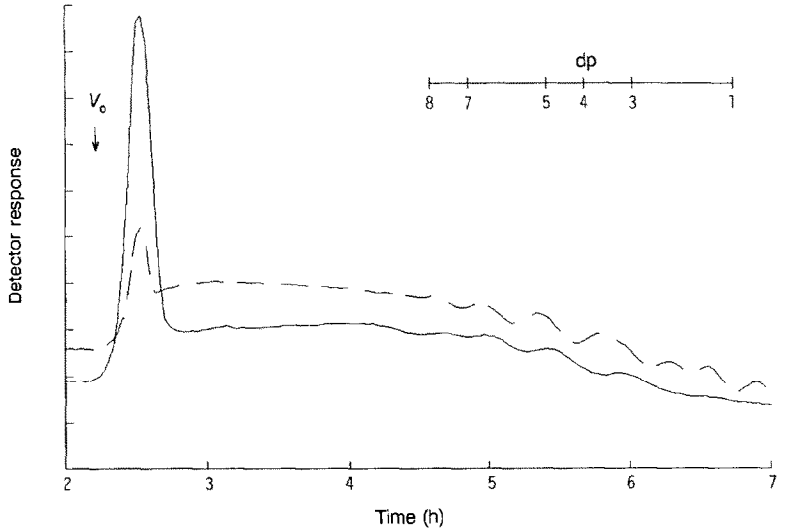


Fig. 3. Gel chromatography of fructoglucan fraction III (———) and of Polydextrose™ (— — —) on Bio-Gel P-2. Calibration with maltodextrins, RI detection.

the sample was divided. Fraction III was further subjected to chromatography on Bio-Gel P-2 and again compared with Polydextrose™ (Fig. 3). The effect of mild-acid hydrolysis of fraction III is shown in Fig. 4, using the assay for total anhydrohexose as described later. This demonstrates the expected decrease in dp. However, a further interesting effect of hydrolysis was observed in the actual

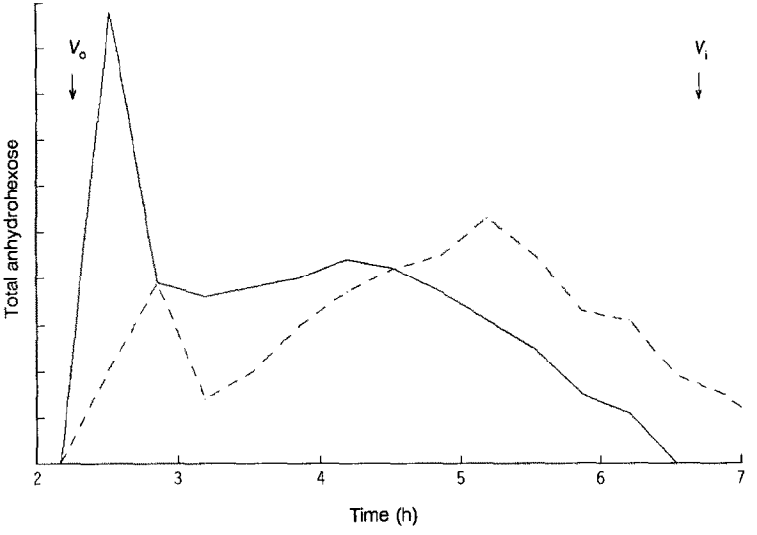


Fig. 4. Gel chromatography of fraction III of fructoglucan on Bio-Gel P-2 before (———) and after (— — —) mild hydrolysis; detection as total anhydrohexose.

TABLE III

Analysis of fractions obtained from preparative gel chromatography of polymer product [a]

Fraction	Total anhydrohexose [b] (mg)	Ratio [c] Glc:Fru	A_{450}
I	3	2:1	17.5
II	4	1.5:1	8.0
III	39	1.7:1	1.1

[a] Conditions used: 80 min, 170°C, air. [b] See Experimental, since 45 mg loaded, this represents 102% yield. [c] Combined glucose and fructose from assay gave 96% anhydrohexose, the same result was obtained from phenol–sulfuric acid testing of the entire polymer.

chromatogram of the hydrolyzate using refractive-index (RI) detection. At the minimum on the total anhydrohexose curve, which occurs just after 3 h, an intense and broad peak appeared on the chromatographic trace. As this peak contained very little carbohydrate material, it may represent a citrate ester, which has not been further investigated. This material evidently has a very large RI response, since citric acid was only present in the original material at 1% by weight, and we have confirmed a high RI response for citric acid itself. A similar peak was observed upon mild hydrolysis of fraction I and this peak disappeared after treatment of the hydrolyzate with sodium borohydride, which has been used to effect the reduction of sugar esters[6]. Citric acid had a similar retention time to be unknown on Bio-Gel P-6 and also showed an exaggerated RI response, but when both were examined by LC (Waters RP 18, H_2O, 1 mL/min); the retention time of citric acid (5.1 min) differed from that of the unknown (2.5 min). The exact cause of this RI peak, therefore, remains unknown.

The fractions obtained from preparative gel chromatography were tested for visible color, total anhydrohexose content, and glucose-to-fructose ratio (Table III). It was not possible to obtain the total anhydrohexose directly by phenol–sulfuric acid analysis because fructose and glucose give different responses. Therefore, an indirect method was used. The fructose content was assayed by means of the cysteine–carbazole test and a projected result due to fructose subtracted from the phenol–sulfuric acid assay of the whole polymer, the difference thus being the absorption due to glucose. The results show that most of the color (though a minor portion of the weight yield) resides in the higher molecular-weight fraction and is probably due to polymeric products of fructose degradation.

Methylation analysis of fructoglucan.—The methylation analysis of the fructoglucan presented problems. Firstly, the random nature of the polymerization leads to a wide variety of linkage types[3c] and consequently of methylation-analysis products. Further complexity arises from the presence of both glucose and fructose pyranose and furanose residues. A further problem associated with the presence of fructose occurs when partially methylated alditol acetates are used for linkage identification. It is not possible to distinguish by GLC between the tri-*O*-methyl

derivatives arising from fructofuranoside residues linked at C-2 and C-1 and those linked at C-2 and C-6. It is, however, possible to distinguish the mass spectra of these when borodeuteride is used for the reduction step[7].

A far more serious problem relates to the method used to cleave the methylated polysaccharide. Hydrolysis conditions which adequately cleave pyranoside linkages also result in total decomposition of the fructose residues, whereas methods that preserve the fructose derivatives do not adequately cleave all of the linkages present[8].

Reductive cleavage cleaves both types of linkage without degradation of fructose derivatives[9] and may merit consideration for this type of polysaccharide. Nevertheless, taking into consideration the likelihood of the occurrence of a wide variety of linkages types, the partially methylated alditol acetates, with their readily interpretable spectra, were chosen and a compromise hydrolysis procedure was sought.

Three hydrolysis procedures were employed, the details of which are outlined in the experimental.

Mild hydrolysis.—(15% acetic acid, 60 min, 100°C). This method gave complete cleavage of methylated sucrose and partial cleavage of methylated raffinose to give the fructose derivative and the 6-*O*-galactopyranosylglucitol derivative. It was expected that this type of hydrolysis would cleave the methylated fructoglucan into smaller fragments, but would only liberate monosaccharides derived from terminal furanosides.

Strong hydrolysis.—(15% trifluoroacetic acid (TFA), 60 min, 120°C). This method gave complete cleavage of methylated sucrose and raffinose, with total decomposition of fructose residues. It was expected that this method would allow assessment of the types of glucose residues present and their relative ratios.

Moderate hydrolysis.—(1.5% TFA, 60 min, 120°C). This method gave almost complete cleavage of methylated stachyose. About 12% (by weight) of the 6-*O*-galactopyranosylgalactitol derivative remained. Loss of fructose derivative due to acid degradation was ~ 10% by effective carbon response (ECR)[10]. In addition, up to 15% (by ECR) of each tetra-*O*-methyl derivative was lost during work-up. It is suspected that these losses occur during evaporation of aqueous solutions after acid hydrolysis and before acetylation.

Similar results were obtained with methylated raffinose, but a 20% loss of fructose occurred from methylated sucrose. Nevertheless, it was accepted that, although this method was not quantitative, it would provide a useful *qualitative* idea of the types and proportions of the fructose residues.

It was anticipated that the highly branched nature of the polymer would make it resistant to methylation (see refs. 3d and 11). Therefore, the fructoglucan was subjected to a series of methylations followed by strong hydrolysis, reduction with borodeuteride, acetylation, and analysis of components by GLC–MS. These results are outlined in Table IV. They show that the relative yields of mono- and di-*O*-methyl products (which are derived from the most hindered glucose units in

TABLE IV

The effect of repeated methylations on the analysis products of fructoglucan [a] after strong hydrolysis

Partially methylated alditol acetate	Products [b] present (%) after n methylations			
	$n=1$	$n=2$	$n=4$	$n=6$
2,3,4,6-Me_4Glc$p$ [d]	167	139 [c]	164	167
2,3,4,5-Me_4Glc$f$ [d]	22	19	21	23
3,4,6-Me_3Glc$p$	28	26	28	27
2,4,6-Me_3Glc$p$	15	10	11	11
2,3,6-Me_3Glc$p$	29	28	29	29
2,3,4-Me_3Glc$p$	100	100	100	100
2,3,5-Me_3Glc$f$	12	15	14	14
2,6-Me_2Glc$p$	16	11	8	8
2,3-Me_2Glc$p$ [d]	35	41	34	25
3,4-Me_2Glc$p$ and 2,4-Me_2Glc$p$ [d]	36	34	35	29
2-MeGlc$p$	21	12	8	7
1,2,3,4,5,6-Ac_6Glc$p$	7	4	3	3

[a] Conditions used:80 min, 170°C, air. [b] Average of four injections, expressed as a percentage relative to 2,3,4-Me_3Glc$p$ (by ECR[10]). [c] Probably some loss by volatilization. [d] These peaks were difficult to resolve and gave variable results.

the polymer) become constant after four methylations. Accordingly, six successive methylations were routinely used in analysis of the polymer.

The per-*O*-methylated polymer was hydrolyzed by the mild, strong, and moderate methods and the partially methylated alditol acetates resulting from all procedures were analyzed by GLC–MS. Very little material amenable to GLC was produced by the mild reaction. In fact, one of the most prominent peaks was that of hexa-*O*-methylhexitol resulting from free glucose. Free glucose had previously been shown to constitute 1–2% of the total precipitable material by phenol–sulfuric acid testing of the appropriate GPC fraction. Similar amounts of 2,5-di-*O*-acetyl-1,3,4,6-tetra-*O*-methyl-(2-^{2}H)hexitol and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-(1-^{2}H)hexitol were found. These two residues would result from a sucrose end-group linked, respectively, through glucose or fructose. These results indicate that most of the fructose is bonded by pyranoside linkages. These pyranoside linkages must be due to glucose as no evidence of fructopyranoside was found in subsequent methylation analyses. This confirms that the product is a fructoglucan and not composed of separate fructan and glucan components. The results for the strong and moderate hydrolyses are outlined in Tables V and VI and a quantitative analysis given in Table VII. The following points should be noted.

(*a*) Because of the nature of the mass-spectral fragmentation, it is not possible to distinguish, even with borodeuteride reduction, the derivative arising from 1,2-linked fructofuranosyl and 1,2-linked glucopyranosyl residues. However it may reasonably be assumed that the difference between the products of the strong and moderate procedures would be due to fructose derivatives.

(*b*) Derivatives arising from 1,2- and 2,6-linked fructofuranosyl residues cannot be resolved by GLC. However the borodeuteride reduction results in different

TABLE V

Products of methylation analysis of fructoglucan using six-fold methylation and strong hydrolysis conditions

T_R [a,b]	Identity	Origin
0.85	6-*O*-acetyl-1,2,3,4,5-penta-*O*-methyl-(1-^{2}H)hexitol	glucose reducing end group
1.00	1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-(1-^{2}H)hexitol	terminal nonreducing glucopyranose
1.01	1,4-di*O*-acetyl-2,3,5,6-tetra-*O*-methyl-(1-^{2}H)hexitol	terminal nonreducing glucofuranose
1.14	1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-(1-^{2}H) hexitol	1,2-linked glucopyranose
1.15	1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-(1-^{2}H) hexitol	1,3-linked glucopyranose
1.17	1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-(1-^{2}H) hexitol	1,4-linked glucopyranose [c]
1.21	1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-(1-^{2}H) hexitol	1,6-linked glucopyranose
1.24	1,4,6-tri-*O*-acetyl-2,3,5-tri-*O*-methyl-(1-^{2}H) hexitol	1,6-linked glucofuranose
1.30	1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-(1-^{2}H)hexitol	1,3,4-linked glucopyranose [c]
1.39	1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-(1-^{2}H)hexitol	1,4,6-linked glucopyranose [c]
1.40	1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methyl-(1-^{2}H)hexitol and 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-(1-^{2}H)hexitol	1,3,6-linked glucopyranose and 1,2,6-linked glucopyranose
1.53	1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-(1-^{2}H)hexitol	1,3,4,6-linked glucopyranose [c]

[a] Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-(1-^{2}H)hexitol from terminal glucopyranose. [b] GLC conditions: Hewlett–Packard Ultra 2 (25 m×0.31 mm) 55°C, 2 min, 30°C/min to 180°C, 2°C/min to 320°C and hold. [c] A furanose alternative exists but is thought unlikely.

fragmentation patterns. The mass spectrum of the augmented peak at t_R 1.15 after moderate hydrolysis was unchanged from that noted under strong hydrolysis, indicating that 1,2-linked fructofuranosyl residues predominate.

(*c*) Derivatives arising from 1,2- and 1,3-linked glucopyranosyl residues could be resolved after strong hydrolysis. However after moderate hydrolysis the increased size of the former peak prevented resolution.

(*d*) The 3,4- and 2,4-di-*O*-methyl substituted hexitols arising from glucose were not resolvable but, after strong hydrolysis, the spectrum showed characteristics of both compounds. After moderate hydrolysis the spectrum showed predominantly a 3,4-di-*O*-methyl substituted hexitol, indicating that the fructose derivative was from a residue linked at positions 1, 2, and 6.

These results show that the fructoglucan is indeed highly branched, and repeated treatments are required to ensure complete methylation. The glucose-to-fructose ratio of the combined products using moderate hydrolysis is ~5:1, which is considerably different from the results in Table III. This may be due in part to

TABLE VI

Products of methylation analysis of fructoglucan using six-fold methylation and moderate hydrolysis conditions

$T_R^{a,b}$	Identity	Origin
0.85	6-*O*-acetyl-1,2,3,4,5-penta-*O*-methyl-(1-^{2}H)hexitol	glucose reducing end group
0.93	2,5-di-*O*-acetyl-1,3,4,6-tetra-*O*-methyl-(2-^{2}H)hexitol	terminal nonreducing fructofuranose
1.00	1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-(1-^{2}H)hexitol	terminal nonreducing glucopyranose
1.01	1,4-di-*O*-acetyl-2,3,5,6-tetra-*O*-methyl-(1-^{2}H)hexitol	terminal nonreducing glucofuranose
1.15	1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-(?-^{2}H)hexitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-(1-^{2}H)hexitol	1,2-linked fructofuranose or glucopyranose and 1,3-linked glucopyranose
1.17	1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-(1-^{2}H)hexitol	1,4-linked glucopyranose [c]
1.21	1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-(1-^{2}H)hexitol	1,6-linked glucopyranose
1.24	1,4,6-tri-*O*-acetyl-2,3,5-tri-*O*-methyl-(1-^{2}H)hexitol	1,6-linked glucofuranose
1.29	1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-(1-^{2}H)hexitol	1,3,4-linked glucopyranose [c]
1.39	1,4,5,6-tetra-*o*-acetyl-2,3-di-*O*-methyl-(1-^{2}H)hexitol	1,4,6-linked glucopyranose
1.40	1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-(1-^{2}H)hexitol, 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-(1-^{2}H) hexitol, and 1,3,4,6-tetra-*O*-acetyl-2,4-di-*O*-methyl-(1-^{2}H)hexitol	1,2,6-linked fructofuranose, 1,2,6-linked glucopyranose and 1,3,6-linked glucopyranose
1.53	1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-(1-^{2}H)hexitol	1,3,4,6-linked glucopyranose [c]

[a] Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-(1-^{2}H)hexitol from terminal glucopyranose. [b] GLC conditions as for Table I. [c] A furanose alternative exists but is thought unlikely.

the loss of fructose derivatives during hydrolysis, as already mentioned. An alternative explanation might be the presence of difructose dianhydride linkages. Difructose dianhydrides have been identified in the oligosaccharide component of the polymerization, and one of these has proved to be resistant to moderate hydrolysis when permethylated *. Disaccharide material was observed in the GLC trace of the moderate hydrolysis sample from methylation analysis of the fructoglucan, and higher-molecular-weight material may be present which is not amenable to GLC. Most of the fructose residues identified are internal, linked through positions 1 and 2, or as 1,2,6-linked branch points. It was expected, on steric grounds, that most of the linkages would be through primary positions and this is

* Moderate hydrolysis of permethylated D-fructofuranose-1,2′:2,1′-D-fructopyranose dianhydride yielded 50% (GLC response by weight) of unhydrolyzed material.

TABLE VII

Results of methylation analysis of fructoglucan using six-fold methylation, and strong and moderate hydrolyses

Derivative	Strong hydrolysis [a,b]	Moderate hydrolysis [a,c]
1,2,3,4,5-Me_5Glc*p*	11	15±1
1,3,4,6-Me_4Fru*f*		5±1
2,3,4,6-Me_4Glc*p*	177	157±1
2,3,5,6-Me_4Glc*f* [d]		23±3
3,4,6-Me_3Glc*f* and 3,4,6-Me_3Fru*f*	28	91±3
2,4,6-Me_3Glc*p*	11	[e]
2,3,6-Me_3Glc*p*	29	19±2
2,3,4-Me_3Glc*p*	100	100
2,3,5-Me_3Glc*f*	14	13±2
2,6-Me_3Glc*p*	9	13±1
2,3-Me_2Glc*p* [d]	30	38±6
3,4-Me_2Glc*p*, 3,4-Me_2Fru*f*, and 2,4-Me_3Glc*p* [d]	29	57±12
2-MeGlc*p*	8	9±1

[a] Average of four injections expressed as a percentage relative to 2,3,4-Me_3Glc*p* (by ECR[10]). [b] Average of two experiments. [c] Mean and standard deviation of three experiments. [d] These peaks were difficult to resolve and gave variable results. [e] Not determined since it was unresolved from the 3,4,6-Me_3Glc*f* and 3,4,6-Me_3Fru*f* peaks.

confirmed by the results for glucose. However, a wider variety of linkages is observed with glucose and it is possible that a small percentage of fructose residues having other types of linkage are lost in background material. The results indicate that every third residue is branched in some way, and this concurs with studies of thermally polymerized glucose[3c].

The presence of glucose reducing-end groups merely indicates the proportion of polymer molecules that started from attack upon free glucose. The molecular weight is not accessible through reducing-end-group analysis, as not every molecule possesses a reducing-end group. Difructose dianhydrides, sucrose, and levoglucosan[12] may all terminate a molecule instead of a reducing-end group. Levoglucosan has also been identified in the oligosaccharide component.

CONCLUSIONS

The polymer is highly branched and of low molecular weight, with an average dp of ~ 25 for the major portion. It contains both fructose and glucose in the ratio ~ 1:2. Most of the nonreducing end groups are glucose units, of which ~ 12% are furanose, with a small proportion of fructofuranose units. The "reducing-end groups" include some glucopyranose units linked at the 6-position, plus possibly levoglucosan and difructose dianhydride units. Almost all of the fructose units are

internal, they are predominantly in the furanose form and are generally linked at the 1-position, with about one-third of these also providing branch points at the 6-position. The internal glucose units are predominantly pyranose, linked mostly through the 6-position, with some also providing branch points at the 2-, 3-, and 4-positions. Some glucose units are doubly branched with substituents at 3-, 4-, and 6-positions of the same glucose unit. On the basis of model experiments with sucrose and simple alcohols[13], we assume that both α and β linkages are present.

EXPERIMENTAL

Preparation of polymers.—Anhydrous, amorphous, acidified sucrose (6.00 g, containing 1% citric acid) was prepared according to the procedure outlined in ref. 1. Samples were heated in a thermostatted oven at the temperatures ($\pm$1°C) and for the times indicated in Table I. Where it is indicated that the sample was heated in a vacuum, the sample was connected to a vacuum pump (15 mmHg) before heating commenced. In cases where air is indicated, the sample was heated in a glass recrystallization dish, open to the air within the oven. The acidified sucrose, which was in the form of a solid white foam, rapidly collapsed upon heating to give a viscous liquid. Any trace of residual water entrained in the glass was evolved as bubbles very early in the heating procedure. Later bubble formation was attributed to the evolution of gaseous products. Color formation commenced early in the heating procedure and was more marked in the higher-temperature samples. The color deepened with time. Upon removal from the oven, the mixture solidified almost immediately to give a walnut-colored glass, which cracked upon standing. The glassy product was dissolved in aq ammonia (14 mL, 0.1 M) and added dropwise with vigorous stirring to abs. EtOH (266 mL). The resulting precipitate was refrigerated overnight before centrifuging. The supernatant, which contained the oligosaccharide components, was retained for further study. The precipitate was washed with abs EtOH (3 × 100 mL), acetone (2 × 100 mL), and dried in a vacuum (< 40°C) to a pale-beige powder.

Measurement of color.—Visible color was measured with a Hewlett–Packard HP 8452A diode array spectrophotometer using 0.1% solutions and normalized to 1% as shown in Table 1.

Gel chromatography.—Three gels were used under the following conditions. Sephadex G-100-120 (bead size 40–120 μm), the column (350 × 26 mm i.d.) was pumped continuously with deionized water at 0.25 mL/min using a Waters 590 programmable LC pump. A Waters R401 differential refractometer was used for detection, and the signal was monitored and accumulated using a Hewlett–Packard Vectra 286 personal computer fitted with a PCL-711S A/D converter. (Program developed by Dr T.T. Stevenson.) For analytical purposes, 8-mg samples were loaded using a 2-mL loop on a Rheodyne 7125 injection valve, and for preparative purposes a 45-mg sample was loaded.

Bio-Gel P-6 Fine 65 μm (Bio-Rad), a 620 × 16 mm i.d. column was similarly pumped at 0.16 mL/min. For analytical purposes a 10-mg sample size was loaded.

Bio-Gel P-2 minus 400 mesh (Bio-Rad), a 580 × 16 mm i.d. column was similarly pumped at 0.25 mL/min. For analytical purposes a 5-mg sample was loaded.

Commercial Polydextrose™ was kindly provided by Pfizer Pharmaceuticals.

Mild acid hydrolysis of polymer fractions.—Fraction III (5.0 mg) was dissolved in 1 M HOAc (1.8 mL) and heated in an oil both for 60 min at 90°C in a Teflon-lined, screw-capped vial. These conditions had previously been shown to hydrolyze sucrose completely. The hydrolyzate was taken to dryness in a vacuum (< 40°C) and redissolved in water for chromatography. Fraction I was similarly treated, and after chromatography the combined effluent from the column was taken to dryness in a vacuum (< 40°C), redissolved in water (250 μL), and treated with 2.5 mg of $NaBH_4$ in water (250 μL) for 120 min at 25°C. The mixture was acidified by treatment with freshly washed IRC-50 resin and then evaporated to dryness in a stream of dry air (i.e., "blown dry") at < 40°C. The sample was blown dry repeatedly with MeOH (7 × 1 mL) and dissolved in water for chromatography.

Analysis of total anhydrohexose.—Fructose content was assayed using the cysteine–carbazole–H_2SO_4 method of Dische[14], and from this a projected response due to fructose in the phenol–H_2SO_4 test[15] was estimated. This estimate was subtracted from the response of the total polymer to the phenol–H_2SO_4 test giving the response due to glucose. The individual fructose and glucose values were summed to give the total anhydrohexose.

Methylation analysis of the fructoglucan.—Samples (5 mg in 250 μL water) were heated for 5 min at 110°C to ensure dissolution of the high-molecular-weight component. Upon cooling the samples were reduced by treatment with $NaBD_4$ (8 mg) in water (400 μL) for 120 min at 25°C. The solutions were acidified with freshly-washed Amberlite IRC-50 resin and blown dry at < 40°C. The residues were further blown dry repeatedly with MeOH (6 × 1 mL) and then dissolved in dry Me_2SO (1 mL) in a Teflon-capped vial. Methylation was carried out using the method of Ciucanu and Kerek[16]. Powdered NaOH (100 mg) was added, the mixture was stirred for 5 min, and then cooled in ice–water. Iodomethane (250 μL) was added and the mixture stirred for a further 40 min. Excess MeI was blown off under a stream of dry N_2 and then water (2 mL), and $CHCl_3$ (2 mL) were added, and the tubes shaken. The $CHCl_3$ layer containing the methylated polysaccharide was washed with water (3 × 2 mL) and placed in a freezer overnight to freeze out any residual water. In the terminology of this paper, the procedure just described is termed a single methylation. A double methylation was carried out by adding a second amount of NaOH and MeI after the original mixture had been stirring for 20 min. Four- and six-fold methylations were carried out by extracting and washing the sample between each double methylation. For hydrolysis the methylated samples were blown dry and subjected to one of the following hydrolysis procedures.

Strong hydrolysis.—Methylated samples were dissolved in 90% (TFA, 200 μL) and heated in a Teflon-capped vial for 10 min at 120°C. Water (1000 μL) was added and the sample heated for a further 60 min at 120°C. The hydrolyzed samples were blown dry (< 40°C).

Mild hydrolysis.—The procedure was as for the strong hydrolysis but HOAc was used instead of TFA and heating was at 100°C.

Moderate hydrolysis.—Methylated samples were dissolved in 90% TFA at room temperature in a test tube (150 μL, no more than 20 s), water (8.85 mL) was immediately added, and the tubes then sealed and heated for 60 min at 120°C. The tubes were cooled and the contents evaporated to dryness in a vacuum (< 40°C). Samples were reduced and then acetylated using the method of Blakeney et al.[17].

Partially methylated alditol acetates were identified by GLC–MS using a Hewlett–Packard 5890A gas chromatograph fitted with a Hewlett–Packard Ultra 1 (25 m × 0.2 mm) crosslinked phenyl methyl silicone–fused-silica capillary column and a Hewlett–Packard 5970 series mass-selective detector (70 eV ionizing potential). Chromatography conditions were 80°C, 2 min, 30°C/min to 200°C, hold for 2 min, and 4°C/min to 320°C.

Quantitation was achieved using GLC–FID on a Hewlett–Packard 5890A gas chromatograph fitted with a Hewlett–Packard Ultra 2 (25 m × 0.33 mm) crosslinked phenyl methyl silicone–fused-silica capillary column. Gas chromatography conditions were as outlined in the text.

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