Carbohydrate Research, 254 (1994) 195–202
Elsevier Science B.V., Amsterdam

Anhydro sugars and oligosaccharides from the thermolysis of sucrose

Merilyn Manley-Harris and Geoffrey N. Richards
Wood Chemistry Laboratory, University of Montana, Missoula, Montana 59812 (USA)

(Received June 17th, 1993; accepted August 20th, 1993)

ABSTRACT

Thermolysis of anhydrous, amorphous, acidified sucrose results in polymerization initially involving the fructosyl cation and later the glucosyl cation. Monomeric and dimeric anhydro sugars form during the thermolysis and are incorporated into the fructoglucan polymer.

INTRODUCTION

The heating of anhydrous, amorphous, acidified sucrose produces a fructoglucan which has been characterized[1]. This polymer is built up by nucleophilic attack of hydroxyl groups upon fructosyl cations arising from scission of sucrose and upon glucosyl cations arising from glucose, which is the other product of this scission[1].

The ethanol-soluble fraction remaining after precipitation of the polymer contains material of lower molecular weight and is referred to herein as sucrose thermal oligosaccharides (STO). The STO have been investigated and found to include a number of anhydro sugars, including two di-D-fructose dianhydrides. The latter have been isolated and identified. Some di-D-fructose dianhydrides have previously been identified, together with many glucose disaccharides, in commercial caramels[2]. Identification in that case was achieved through high-resolution MS, which yielded an exact molecular weight; actual structures were not obtained.

RESULTS AND DISCUSSION

The STO were obtained by evaporation of the supernatant remaining after precipitation of the fructoglucan[1] and were fractionated by gel chromatography (Fig. 1). In Fig. 1 the maltodextrins are included only as an approximate guide to degree of polymerisation (dp)[1]. Yields, glucose:fructose ratios, and total hexose contents of the individual fractions are reported in Table I.

SSDI 0008-6215(93)E0301-G

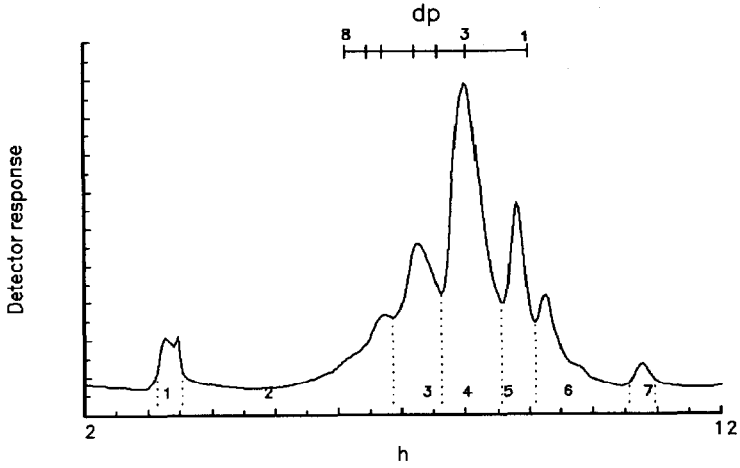


Fig. 1. Fractionation of STO on Bio-Gel P-2. Calibration with maltodextrins, RI dectection.

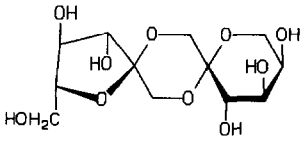
Fraction 1 contains residual citric acid, which gives an exaggerated RI response, and possible traces of polymer. Fraction 7 is 5-hydroxymethylfurfural, which gives positive results with the colorimetric tests used for hexoses in this assay. LC revealed glucose in fraction 5 and, in fraction 6, 1,6-anhydrogluco-pyranose and -furanose. This was confirmed by GLC–MS of the *O*-trimethylsilyl derivatives. LC of fraction 4 showed a cluster of disaccharides in which two species, **1** and **2**, predominated. Compounds **1** and **2** were tentatively identified as di-D-fructose dianhydrides on the basis of LC and GPC retention times and were isolated by preparative LC (Fig. 2). Methylation analysis and comparison of the ^{13}C NMR spectrum with the literature[3,4] permitted the identification of **1** as diheterolevulosan II (α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride). Similarly, **2** was identified as difructose anhydride I (α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride).

TABLE I

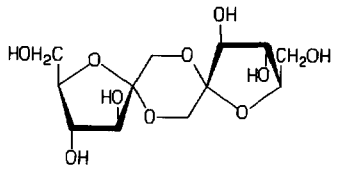
Analysis of fractions of sucrose thermal oligosaccharides (STO)

Fraction	% of supernatant (by weight) [a]	G:F ratio	% apparent anhydrohexose
1	3.1	0.76	41
2	13.8	0.97	97
3	17.5	0.66	100
4	30.9	0.44	100
5	13.1	1.55	100
6	12.2	1.54	100
7	1.3	0.41	33

[a] Total recovery 92% by weight.



1



2

Quantitation of the individual components was achieved by GLC–FID of the trimethylsilyl derivative of the total STO (Fig. 3). The results are shown in Table II. Mass-spectral fragmentation patterns of the disaccharides other than **1** and **2** indicated the presence of three other di-D-fructofuranose dianhydrides, one other D-fructofuranose-D-fructopyranose dianhydride and a di-D-fructopyranose dianhydride[5]. However it was not possible to isolate these for characterization. The predominance of **1** and **2** is expected in terms of their stabilities[4]; indeed we have found **1** to be exceptionally stable to the conditions of moderate hydrolysis[1] used

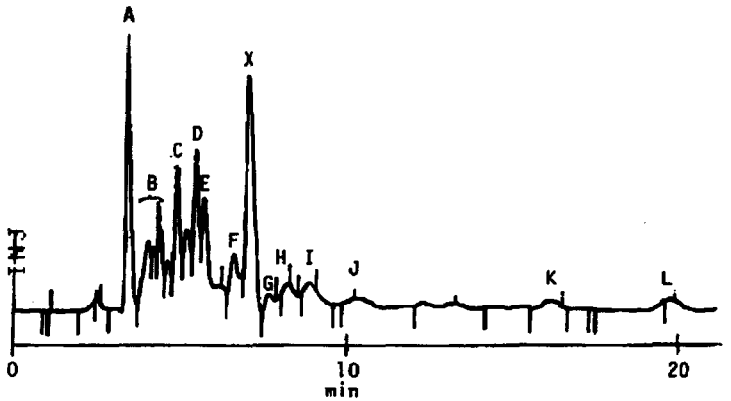


Fig. 2. LC separation of components of STO.

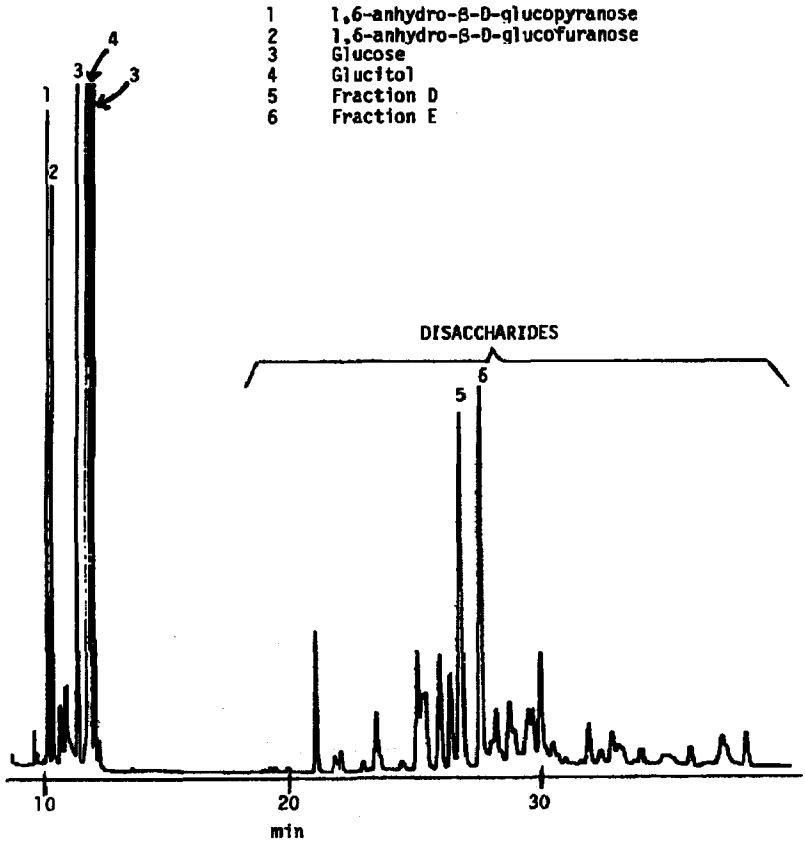


Fig. 3. GLC-FID of trimethylsilyl derivative of STO (Utra 2, 55°C (2 min)+30°C/min → 230°C+1°C/min → 320°C).

in the methylation analysis. In **1** the pyranose and dioxane rings are both in chair conformations and the furanose ring is in an envelope conformation, in conformity with expectations from the anomeric effect[6]. Deslongchamps has indicated that,

TABLE II

Quantitation of components of sucrose thermal oligosaccharides (STO)

Component	% of STO [a]
Glucose	7.3
1,6-Anhydro-D-glucofuranose	1.6
1,6-Anhydro-D-glucopyranose	2.0
1	3.9
2	4.1
Total disaccharides, including **1** and **2**	18.9

[a] Response factors relative to glucitol: glucose 0.70; 16-anhydro-D-gluco-furanose and -pyranose 0.72; disaccharides, including **1** and **2**, 0.57.

TABLE III

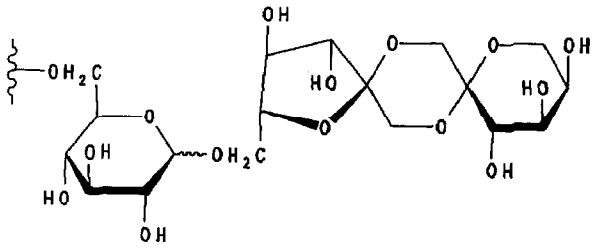
Methylation analysis of GPC fraction 2 compared with fructoglucan polymer [a]

Derivative	Strong hydrolysis		Moderate hydrolysis	
	Fraction 2	Fructoglucan	Fraction 2	Fructoglucan
1,2,3,4,5-Me_5Glc	11	11	22	15
1,3,4,6-Me_4Fru*f*			15	5
2,3,4,6-Me_4Glc*p* [b]	265	177	242	157
2,3,5,6-Me_4Glc*f* [b]	69	23	85	23
3,4,6-Me_3Glc*p* and 3,4,6-Me_3Fru*f*	33	28	226	91
2,4,6-Me_3Glc*p*	24	11	ND [c]	ND [c]
2,3,6-Me_3Glc*p*	43	29	36	19
2,3,4-Me_3Glc*p*	100	100	100	100
2,3,5-Me_3Glc*f*	28	14	33	13
2,6-Me_2Glc*p*	24	9	38	13
2,3-Me_2Glc*p* [b]	52	30	ND [c]	38
3,4-Me_2Glc*p* and 3,4-Me_2Fru*f* and 2,4-Me_3Glc*p* [b]	35	29	188	57
2-MeGlc*p*	16	8	14	9

[a] Values taken from ref 1. [b] These peaks were difficult to resolve and gave variable results. [c] Not determined, as it was unresolved from the following or preceding peak.

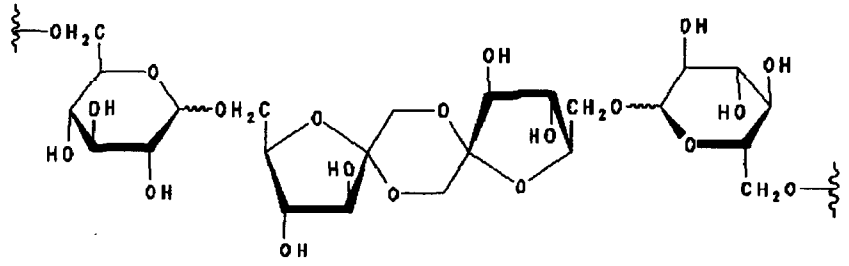
when the system conforms with the anomeric effect, hydrolysis occurs through the ground state[7]. The resistance of **1** to hydrolysis is therefore attributable to the exceptional stability of the ground-state structure.

Fraction 2 of the GPC separation (Fig. 1) was chosen as representative of the oligosaccharides, and a methylation analysis was undertaken using a procedure, involving several different types of hydrolysis, similar to that employed with the fructoglucan[1]. The results are reported in Table III and compared with those for the fructoglucan. The strong hydrolysis (vigorous conditions) results indicate that fraction 2 is highly branched and has a broadly similar distribution of glucose residues to the fructoglucan; the increased proportion of nonreducing glucopyranose groups indicates a lower dp. The moderate hydrolysis result (milder conditions, permitting survival of fructose residues) shows that a far higher proportion of fructose is present in fraction 2 than in the polymer. The oligosaccharides present in fraction 2 may be regarded as representative of an early stage in polymer growth. Therefore, we conclude that polymer formation occurs by the relatively late addition of glucosyl residues to a previously formed, fructose-rich core. This effect is presumably due to the fact that the first reaction in this system is the thermolytic scission of protonated sucrose to yield fructofuranosyl cation (Fru^+) and glucose. Fru^+ then adds to any available primary hydroxyl group, while the glucose is relative inert. In a slower process the glucose can lose its C-1 protonated hydroxyl group to form glucopyranosyl cation, which adds to the already formed oligosaccharides to produce the fructoglucan. This accords with our observation that the glucose : fructose ratios of fructoglucan polymers increase with increasing time of heating at a given temperature.



3

The presence of anhydro sugars in the STO suggests that these may be incorporated into the growing polymer. Certainly a precedent exists for the presence of anhydroglucose end-groups in thermally produced polysaccharides[8]. Di-D-fructose dianhydrides could be incorporated in such structures as **3** and **4**.



4

Stable oligosaccharides of similar structure have been synthesized by Defaye and García Fernández[4]. In ref 4, the oligosaccharides were formed by the condensation of glucosylfructoses through an intermediate fructosyl cation, and a similar mechanism could act in our system. Alternatively fructosyl or glucosyl residues could add to already-formed di-D-fructose dianhydrides.

An attempt was made to demonstrate the presence of di-D-fructose dianhydride components in fraction 2 oligosaccharides. Mild hydrolysis of fraction 2 (containing D-glucitol as an internal standard) under conditions that cleave fructofuranosyl linkages but not dianhydride linkages was followed by treatment with a mixture of enzymes, including α- and β-glucosidase, alpha amylase, and invertase. The latter was included in the event that any fructofuranosyl residues had survived the hydrolysis. Gel chromatography of the enzyme-treated sample allowed isolation of a fraction incorporating the bulk of the disaccharides plus the internal standard. GLC–FID of the *O*-trimethylsilyl derivative of this fraction revealed numerous peaks in the disaccharide region, the combined integration of which corresponded to ~ 4% of the original material. GLC–MS of the sample showed three major

peaks in the disaccharide region, identified by their mass spectra as **1**, **2**, and an isomer of **2**. It should be noted that the figure of 4% represents a minimum, since it cannot be expected that the enzymes would achieve complete degradation of a highly branched structure containing a variety of residues and linkages.

CONCLUSIONS

Thermolysis of anhydrous, amorphous, acidified sucrose results in a polymerization process in which glucosyl and fructosyl residues undergo nucleophilic reaction with hydroxyl groups. In the early stages of the polymerization, reaction mainly involves fructosyl cation, and glucosyl cation subsequently adds to the fructose-rich core. Monomeric and dimeric anhydro sugars are also incorporated into the growing polymer.

Fructose-rich oligosaccharides have a beneficial effect upon gastrointestinal flora. Specifically, they encourage the growth of bifidobacteria in the cecum and colon (for example, see ref. 9). We have succeeded in achieving increased yields of these oligosaccharides in the form of a caramel. This caramel is currently undergoing several types of animal trial as a nutritional supplement and has shown dramatic, beneficial results[10].

EXPERIMENTAL

Preparation of STO.—Thermolysis of anhydrous, amorphous, acidified sucrose was carried out for 80 min at 170°C as described previously[1]. After precipitation of the polymer in 95% EtOH, the supernatant was evaporated under vacuum (< 40°C) to yield the STO as a ginger-coloured, hygroscopic glass ($\sim 57\%$) from sucrose.

General methods.—Gel chromatography, colorimetric assays, hydrolyses, and methylation analysis procedures have all been described previously[1]. LC was carried out with a Waters Resolve C_{18}, 5-μm, 8 mm $\times$ 10 cm, Radial Pak cartridge eluted with water at 0.9 mL/min and a Waters differential refractometer, R401. Preparative LC was carried out on a Waters Delta-Pak, 15-μm, 25 mm $\times$ 30 cm cartridge eluted with water at 10 mL/min. GLC–FID of the trimethylsilyl derivatives was achieved using a Hewlett–Packard 5890A gas chromatograph fitted with a Hewlett–Packard Ultra 2 (25 m $\times$ 0.33 mm) crosslinked phenyl methyl silicone fused-silica capillary column. Conditions used were 55°C for 2 min, 30°C/min to 230°C, and 1°C/min to 320°C. ^{13}C NMR spectra of **1** and **2** were recorded at 75 MHz on a Bruker AM-300 spectrometer at the University of Alberta, using 1,4-dioxane as the external standard. Chemical shifts were assigned by comparison with refs 3 and 4. Enzymes were purchased from Sigma and were used in unbuffered, aqueous solution in such a concentration as to give complete hydrolysis of the specified substrate in < 60 min.

Identification of **1** *and* **2**.—Established procedures were used to obtain authentic samples of diheterolevulosan II[11] and di-D-fructose anhydride I[12]. Methylation

analysis with moderate hydrolysis of **1** yielded 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-(2-^{2}H)hexitol and 1,2,6-tri-*O*-acetyl-3,4,5-tri-*O*-methyl-(2-^{2}H)hexitol, together with ~ 50% of unhydrolyzed material. Similar treatment of **2** yielded only 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-(2-^{2}H)hexitol. The ^{13}C NMR spectra were identical with those reported in ref 3.

Mild hydrolysis and enzymic degradation of GPC fraction 2.—Fraction 2 (5 mg in 250 μL) was spiked with an internal standard of D-glucitol and treated with HOAc (2 M, 250 μL) for 90 min at 90°C and then evaporated to dryness under a stream of dry N_2 (< 40°C). The residue was treated with an aqueous solution (250 μL) containing invertase (EC 3.2.1.26, from yeast, 103 units), alpha-amylase (EC 3.2.1.1, from *Bacillus sp.*, 2325 units), α-D-glucosidase (EC 3.2.1.20, from yeast, 104 units) and β-D-glucosidase (EC 3.2.1.21, from almonds, 27.5 units). The mixture was incubated for 24 h at 37°C, passed through a 45-μm filter, and fractionated on Bio-Gel P-2. The fraction containing glucose, fructose, glucitol, and disaccharides was taken to dryness under vacuum (< 40°C) and trimethylsilylated for GLC–FID and GLC–MS. The total area of peaks in the disaccharide region was 3.9% of the original material, using a relative response factor of 0.57 previously determined with pure **2** and D-glucitol; 6.6% of glucose and 2.4% of fructose were also present. Untreated fraction 2, similarly fractionated, showed 0.2% of unidentifiable peaks in the disaccharide region.

ACKNOWLEDGMENTS

The authors thank Todd Lowary at the University of Alberta for recording the NMR spectra and Alan Blize for experimental assistance. This work was supported by a grant from The Sugar Association, Inc.

REFERENCES

1 M. Manley-Harris and G.N. Richards, *Carbohydr. Res.*, 240 (1993) 183–196.
2 H. Tschiersky and W. Baltes, *Z. Lebensm. Unters. Forsch.*, 189 (1989) 132–137.
3 J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 136 (1985) 53–65.
4 J. Defaye and J.M. García Fernández, *Carbohydr. Res.*, 273 (1992) 223–247.
5 M. Manley-Harris and G.N. Richards, *Carbohydr. Res.*, 226 (1992) 327–330.
6 J.A. Kanters, J. Buitenhuis, J. Kroon, M. Mathlouthi, J.H. van der Maas and B. Lutz, *J. Crystallogr. Spectrosc. Res.*, 20 (1990) 1–8.
7 P. Deslongchamps, *Stereoelectronic Effects in Organic Chemistry*, Pergamon Press, Oxford, 1983, pp. 4–53.
8 G.R. Ponder and G.N. Richards, *Carbohydr. Res.*, 208 (1990) 93–104.
9 H. Hidaka, M. Hirayama, T. Tokunaga, and T. Eida, *Adv. Exp. Med. Biol.*, 270 (1990) 105–117.
10 J.I. Orban, J.A. Patterson, A.L. Sutton and G.N. Richards, presented at the Poultry Science Association Annual Meeting, Michigan State University, July, 1993.
11 H.W. Hilton, *Methods Carbohydr. Chem.*, 2 (1963) 199–203.
12 L.A. Boggs and F. Smith, *J. Am. Chem. Soc.*, 78 (1956) 1878–1880