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Di-D-fructose dianhydrides and related oligomers from thermal treatments of inulin and sucrose

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Abstract

Thermal treatment of anhydrous, acidified sucrose or inulin yields caramels containing monosaccharides and oligomers, predominantly dianhydrides and higher oligomers derived by the addition of glycosyl residues to dianhydrides. Fourteen dianhydrides, most of which comprise two fructose moieties, have been identified by mass spectroscopy of the per-*O*-trimethylsilyl ethers. Thirteen of these dianhydrides have been isolated and characterized; five of the dianhydrides are novel compounds and one of these is a glucose–fructose dianhydride. The dianhydrides and related oligomers are thought to have a prebiotic effect by stimulating the proliferation of bifidobacteria in the large intestine. © 1996 Elsevier Science Ltd.

Keywords: Fructose dianhydrides; Thermolysis of sugars; Inulin thermolysis; Sucrose thermolysis

1. Introduction

During thermal treatment of anhydrous acidified sucrose or of inulin, the fructofuranosyl cation (**1**) is formed [1,2]. This cation can react with nucleophiles such as hydroxyl groups attached to other species present, for example sucrose, fructose and glucose. Under more rigorous conditions other cations, for example glucofuranosyl (**2**) and pyranosyl (**3**) cations, may also form and these too may react with other species.

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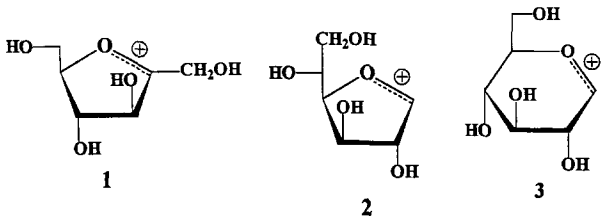


1 2 3

Condensation of two fructose moieties may result in the formation of di-D-fructose dianhydrides and these have been observed previously in thermal treatments of sucrose and of inulin [1,2]. Di-D-fructose dianhydrides have been isolated from a commercial sucrose caramel [3] and from a commercial roasted chicory sample [4]. Defaye and García Fernández [3] also identified, using FABMS and ^{13}C NMR, two series of oligomers in the sucrose caramel, one deriving from glucobioses the other formed by the sequential addition of glucosyl cation to di-D-fructose dianhydrides.

A sucrose caramel, in which the content of fructose-containing oligomers is maximized, has been shown to enhance growth performance of young animals and this is thought to be associated with increased proliferation of bifidobacteria in the large intestines with related beneficial effects upon intestinal health and therefore growth rate of the animal [5]. In order to pursue our understanding of this effect we have attempted to characterize the major constituents of this sucrose caramel and of an inulin caramel produced under similar conditions.

The caramels were found to consist predominantly of monosaccharides and of oligomers, the major fraction of the latter being dimeric dianhydrides. We have now identified thirteen of these dimeric dianhydrides in the thermal product of anhydrous acidified sucrose and all have been isolated and characterized. Five of these are novel compounds, one of which is a glucose–fructose dianhydride. The latter compound is absent in the thermal product from inulin, but the other twelve di-D-fructose dianhydrides are present, together with an extra, as yet unidentified, dianhydride, the presence of which has not been demonstrated in sucrose caramel.

2. Results and discussion

Caramels formed by the thermal treatment of anhydrous acidified sucrose and of inulin were analyzed by size-exclusion chromatography (SEC) the results of which are shown in Fig. 1 and summarized in Table 1.

The SEC fractions were examined by FABMS, electrospray-MS and, where possible, by GCFID and GCMS of the per-*O*-trimethylsilyl derivatives. In both sucrose and inulin products, fraction A contained citric acid and, in the case of inulin, polymeric material excluded by the column. Fraction B consisted of pentamers and higher oligomers, fraction C of tetramers and fraction D of trimers. Fractions E1 and E2 both contained dimers but in the case of inulin caramel these were substantially restricted to the di-D-fructose dianhydrides whereas in sucrose significant amounts of singly-linked

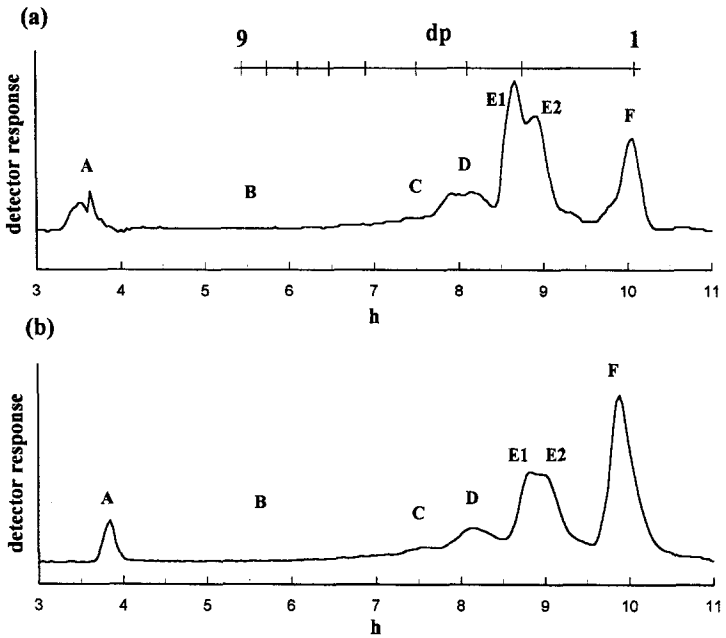


Fig. 1. SEC of inulin (a) and sucrose (b) caramels on Bio-Gel P-2. Calibration by inulooligosaccharides. A–F are discussed in the text.

disaccharides were observed in addition to the dianhydrides. Fraction F contained glucose and fructose and in the case of sucrose caramel the former greatly predominated.

FABMS results.—Results for the FABMS of sucrose caramel were similar to those obtained by Defaye and García Fernández [3]. That is two series of ions were observed arising from successive elimination of mass 162. One series corresponded to oligomers that had formed by successive addition of glycosyl units starting with a dianhydride dimer. The other series corresponded to oligomers containing only singly-linked glycosyl residues. The presence of two molecular ions, differing by 18 mass units, observed

Table 1
Size-exclusion chromatography of inulin and sucrose caramels on Bio-Gel P-2

Fraction	Weight of fraction as a percentage of total material recovered	
	Inulin	Sucrose
A	5.1	1.0
B	4.9	2.7
C	3.9	3.9
D	16.1	14.9
E1	22.6	15.3
E2	26.4	19.4
F	21.0	42.8

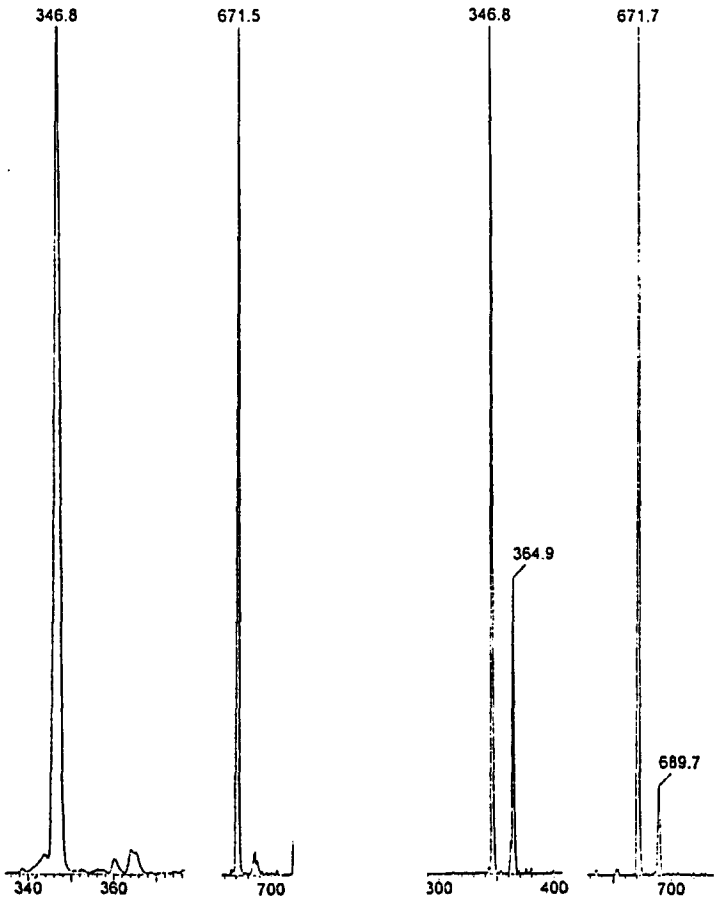
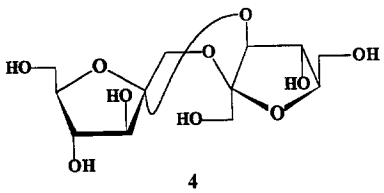


Fig. 2. Electrospray-MS of dimeric and tetrameric SEC fractions of inulin (a) and sucrose (b) caramels. Peaks show the $[M+Na]^+$ ions.

with electrospray technique confirmed this. By contrast the inulin caramel contained only one series of oligomers namely the one deriving from dianhydrides. The difference between the molecular ions observed in the two caramels using electrospray techniques is illustrated in Fig. 2.

Dimer fractions.—The GCFID traces of the per-*O*-Me_3Si derivatives of the unfractionated inulin and sucrose products are shown in Fig. 3. Comparison of the dimer regions revealed twelve peaks corresponding to di-D-fructose dianhydrides in the inulin product whereas in the sucrose product twelve peaks are also present but not the same twelve since sucrose caramel contains an additional dianhydride not present in the inulin caramel while the slowest-running dianhydride peak, **17**, may or may not be obscured by a cluster of singly-linked disaccharides (distinguished by MS, see below). The expanded GCFID traces of the per-*O*-Me_3Si ethers of the dianhydrides (**4–17**) are represented schematically in Fig. 4.



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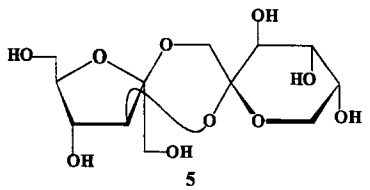

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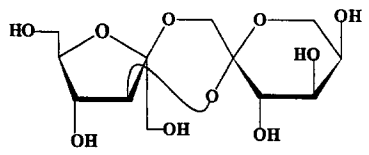

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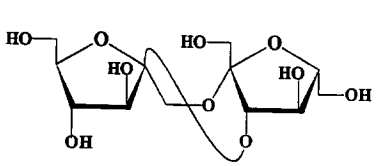

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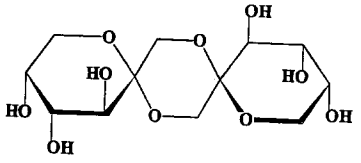

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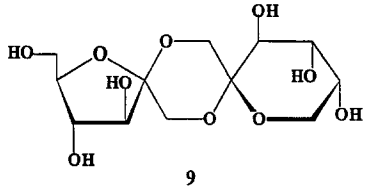

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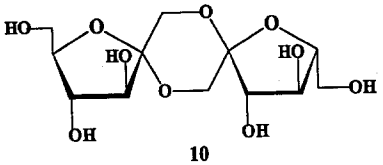

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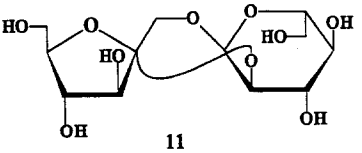

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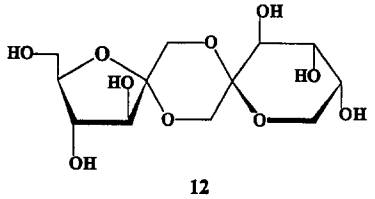

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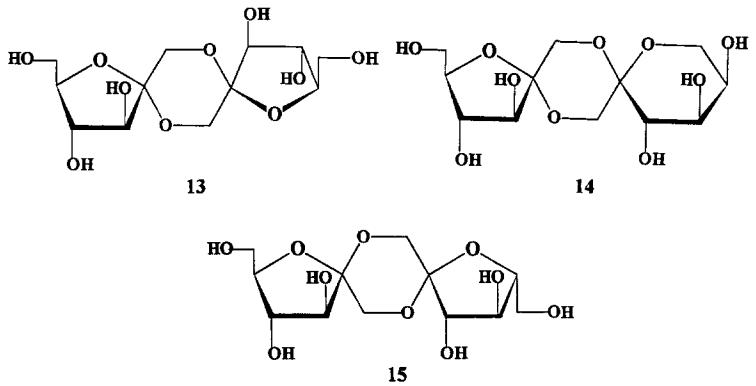


13 14 15

These chromatograms were obtained using a 5% phenyl methyl siloxane capillary column which results in coincidence of peaks for **8** and **9**. Using a methyl siloxane capillary column **8** and **9** could be resolved but peaks for **14** and **15** then became coincident. As indicated below peak **6** contained a trace of second unidentified component, identifiable by mass spectroscopy, besides the dianhydride; neither column resolved these two components. A partial resolution of the dianhydrides was obtained by SEC, **4–6**, **14**, **16** and **17** being found predominantly in dimer peak E2.

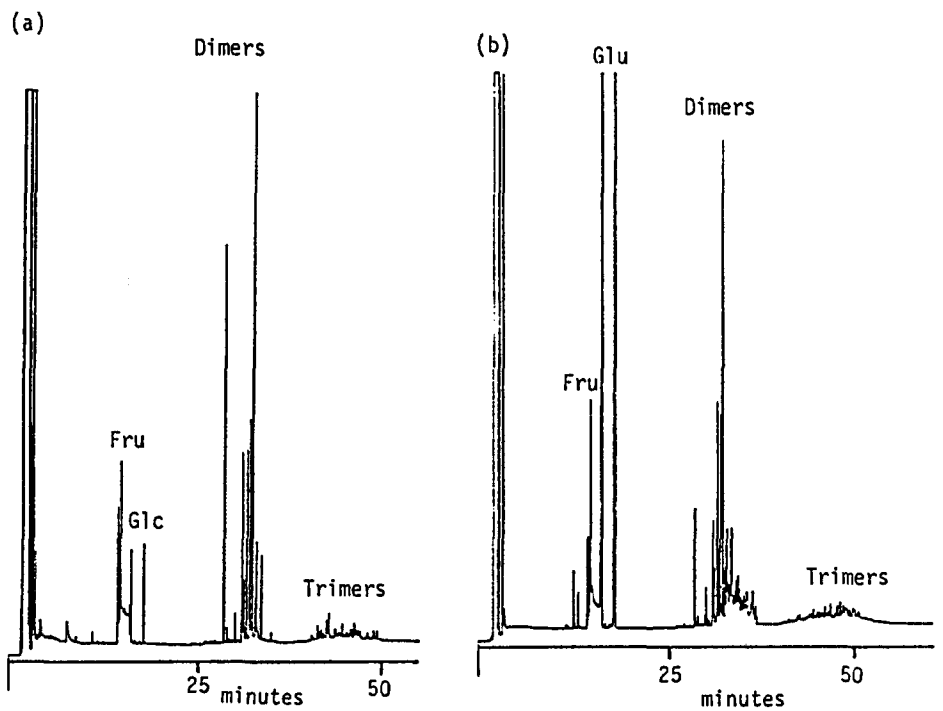
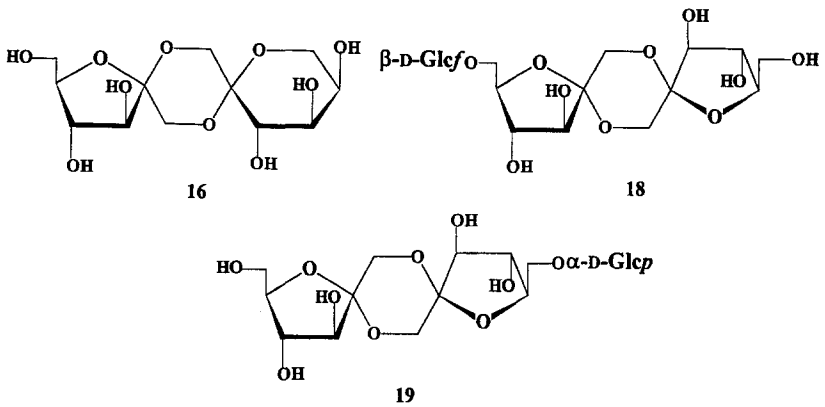


Fig. 3. GCFID of the per-*O*-Me_3Si ethers of inulin (a) and sucrose (b) caramels. Chromatography condiditons are described in the text.



Dianhydrides **4–16** were isolated by successive liquid chromatography (LC) runs, Fig. 5. We have previously isolated **4**, **7**, **12** and **13** from thermal mixtures of inulin [2] and sucrose [1]; they are respectively α-D-fructofuranose β-D-fructofuranose 1,2′:2,3′-dianhydride (difructose anhydride III), di-β-D-fructofuranose 1,2′:2,3′-dianhydride (difructose anhydride II), α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride (di-

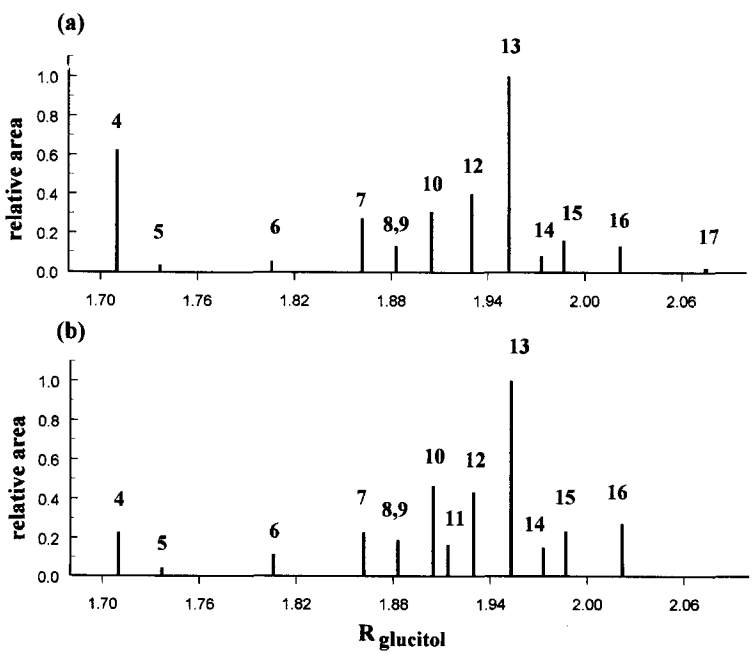


Fig. 4. Schematic representation of the GCFID trace of the per-*O*-Me_3Si ethers of the dianhydrides found in inulin (a) and sucrose (b) caramels.

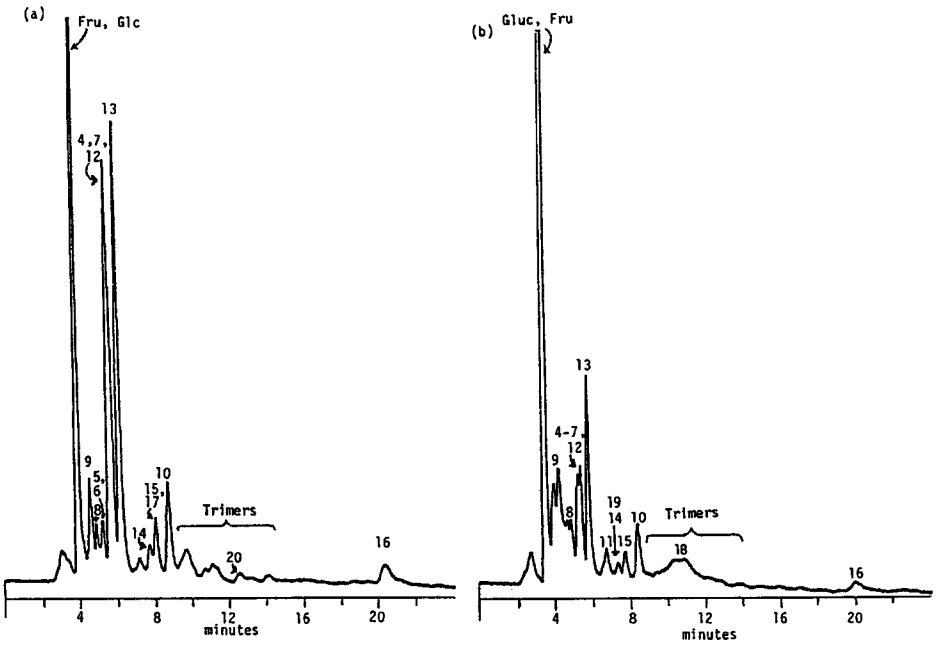


Fig. 5. LC traces of inulin (a) and sucrose (b) caramels. Conditions reverse phase C-18/H_2O/1 mL min^{-1}.

heterolevulosan II) and α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride (difructose anhydride I). Compound **8** was identified by co-injection of the per-*O*-Me_3Si ether on GC with an authentic standard, by its mass spectrum and by its ^{13}C NMR spectrum [6] as α-D-fructopyranose β-D-fructopyranose 1,2′:2,1′-dianhydride (dihetero-levulosan I). Compounds **7**, **8**, **12** and **13** have also been identified in a commercial sucrose caramel [3] and 4, 7 and 13 have been found in commercial roasted chicory [4]. The remaining compounds were characterized by methylation analysis and NMR spectroscopy. ^{1}H and ^{13}C assignments are given in Tables 2 and 3.

Compounds **10** and **15** both contained only 1,2-linked fructofuranose residues by methylation analysis and both showed only 6 peaks in their ^{13}C NMR spectra. This indicates that both are symmetrical 1,2-linked difuranose dianhydrides one having both residues in the α configuration and the other with both in the β configuration. The ^{13}C NMR spectrum of **10** is identical with that of a di-D-fructose dianhydride, formerly assigned tentatively as di-β-D-fructofuranose 1,2′:2,1′-dianhydride [7]. Comparison of the ^{13}C spectra of **10** and **15** with the ^{13}C NMR data for other dianhydrides (Table 2), in particular the shifts of C-2 and C-5, indicates an α configuration for both residues in **10** and a β configuration in **15**. Additional confirmation is obtained from the value of the $J_{3,4}$ coupling constant in the ^{1}H spectra. A range of 1.4–2.4 Hz (mean 2.0 Hz has been found [8] for a series of acetylated α-fructofuranose derivatives, whereas for the β anomers a range of 5.5–9.7 Hz (mean 7.1 Hz) was found. There is a greater possibility of conformational strain in the dianhydrides and the compounds are not acetylated, however the values of $J_{3,4}$ given in Table 2 correspond to this model. The latter is

Table 2
^{1}H NMR spectra of dianhydrides isolated from inulin and sucrose caramels

Compound	Proton chemical shift (ppm) Coupling constant (Hz)													
	H-1_a $J_{1a,1b}$	H-1_b	H-3 $J_{3,4}$	H-4 $J_{4,5}$	H-5 $J_{5,6a}$	H-6_a $J_{5,6b}$	H-6_b $J_{6a,6b}$	H-$1'_a$ $J_{1'a,1'b}$	H-$1'_b$	H-3′ $J_{3',4'}$	H-4′ $J_{4',5'}$	H-5′ $J_{5',6'a}$	H-$6'_a$ $J_{5',6'b}$	H-$6'_b$ $J_{6'a,6'b}$
β-D-Fru*f*-2,1′:3,2′-α-D-Fru*p* (**5**)	3.68 d 12.4	3.62 d	4.31 d 7.2	4.67 dd 7.6	3.64 m 2.4	3.87 dd 5.2	3.71 dd 13.6	4.08 d 13.6	3.73 d	3.71 d 6.4	3.90 dd 3.0	3.96 m	~ 3.94 dd 2.4	3.605 13.6
β-D-Fru*f*-2,1′:3,2′-β-D-Fru*p* (**6**)	4.13 d 12.0	3.50 d	3.96 s ~ 0	4.10 d 1.2	3.94 m	3.71–3.81		3.86 d 11.6	3.73 d	3.54 d 9.6	3.91 dd 3.6	3.99 m	3.67	
β-D-Fru*f*-1,2′:2,1′-α-D-Fru*p* (**9**)	3.98 [a] d 12.4	3.56 [a] d	3.82 d 8.0	4.06 dd 7.2	3.88 m 3.6	3.74 dd 7.2	3.61 dd 12.4	4.07 [a] d 12.4	3.70 [a] dd	3.77 d 5.2	3.86 d 4.2	3.99 m	~ 3.74 dd	3.65 11.2
α-D-Fru*f*-1,2′:2,1′-α-D-Fru*f* (**10**)	3.97 d 12.4	3.79 d	4.14 d 3.2	3.92 dd ~ 6	3.99 m 3.2	3.78 dd 6.0	3.65 dd 12.4							
α-D-Fru*f*-1,2′:2,1′-α-D-Fru*p* (**14**)	3.99 d 12.8	3.80 d	4.22 d 3.6	~ 3.92 dd	4.1 m	3.61 [b] 3.74 [b]	3.63 [b] 3.77 [b]	3.95 d 12.4	3.69 d	3.80 d ~ 4.0	3.88 dd	~ 3.98 m 4.1 m	3.61 [b], 3.63 [b] 3.74 [b], 3.77 [b]	
β-D-Fru*f*-1,2′:2,1′-β-D-Fru*f* (**15**)	3.91 d 12.8	3.98 d	3.97 d 7.6	4.05 dd 7.5	3.84 m 3.4	3.73 dd 6.6	3.61 dd 12.2							
β-D-Fru*f*-1,2′:2,1′-β-D-Fru*p* (**16**)	3.87 d 12.4	3.76 d	4.04 d 7.2	4.05 dd 7.2	3.82 m 4.2	3.72 dd 6.6	3.60 dd 12.2	4.10 d 12.0	3.73 d	3.60 d 10.0	3.84 dd 3.6	3.95 m 1.2	3.89 dd 2.0	3.67 dd 12.8
								H-1 $J_{1,2}$	H-2 $J_{2,3}$	H-3 $J_{3,4}$	H-4 $J_{4,5}$	H-5 $J_{5,6a}$	H-6_a $J_{5,6b}$	H-6_b [c] $J_{6a,6b}$
α-D-Fru*f*-1,1′:2,2′-α-D-Glc*p* (**11**)	4.00 d 12.4	3.55 d	4.00 d 3.2	3.94 dd 6.4	4.03 m 3.0	3.80 dd 6.0	3.64 dd 12.0	5.45 d 2.8	4.19 dd 0.8	4.32 dd 2.8	4.32 dd 10.0	3.85 m 3.2	3.82 dd 5.4	3.70 dd 12.2

[a] It was not possible to distinguish H-1 and H-1′.
[b] It was not possible to distinguish H-6 and H-6′.
[c] Asignments for glucopyranose residue.

Table 3
^{13}C NMR spectra of dimeric dianhydrides and associated trimers isolated from inulin and sucrose caramels

Compound	Carbon chemical shift (ppm)											
	C-2	C-2′	C-3	C-4	C-5	C-3′	C-4′	C-5′	C-6	C-6′	C-1	C-1′
β-D-Fru*f*-2,1′:3,2′-α-D-Fru*p* **(5)**	102.2	97.6	79.8	73.2	81.1	71.3, 71.5		66.1	61.6	62.4	64.2	61.2
β-D-Fru*f*-2,1′:3,2′-β-D-Fru*p* **(6)**	104.6	96.0	85.8	77.1	74.1	69.8	69.8	70.1	63.3	64.4	62.3	64.9
β-D-Fru*f*-1,2′:2,1′-α-D-Fru*p* **(9)**	99.7	95.7	78.0	75.5	82.3	71.7	71.6	65.0	63.7	60.8	63.0, 62.2	
α-D-Fru*f*-1,2′:2,1′-α-D-Fru*f* **(10)**	104.8		81.0	77.8	84.0				62.1		62.2	
α-D-Fru*f*-1,2′:2,1′-α-D-Fru*p* **(14)**	105.4	97.0	80.0	77.8	84.1	71.1	71.4	65.6	62.0, 61.8		59.8	64.0
β-D-Fru*f*-1,2′:2,1′-β-D-Fru*f* **(15)**	101.7		80.2	75.7	82.5				63.4		64.6	
β-D-Fru*f*-1,2′:2,1′-β-D-Fru*p* **(16)**	101.8	97.9	80.8	76.0	82.8	72.1	70.4	70.0	63.5	65.2	63.3	65.8
α-D-Fru*f*-1,1′:2,2′-α-D-Glc*p* **(11)** [a]	103.3	73.4	83.1	78.4	84.1	75.5	81.8	69.9	64.6	62.0	61.7	99.0
Trimers												
β-D-Glc*f*(1 → 6)	80.5		75.9	82.5	70.6			64.6			109.1	
α-D-Fru*f*-1,2′:2,1′-β-D-Fru*f* **(18)**	103.9	99.9	82.5	79.0	83.8	78.0	75.6	82.3	68.6	63.7	62.7	63.6
α-D-Glc*p*(1 → 6′)	72.4		74.0	70.5	73.1				61.5		99.6	
α-D-Fru*f*-1,2′:2,1′-β-D-Fru*f* **(19)**	103.4	100.2	83.0	78.7	84.4	77.8	76.0	80.6	63.6	70.4	62.2	63.1
β-D-Fru*p*(1 → 6′)	101.7		69.7	70.6	70.2				65.1		62.2	
α-D-Fru*f*-1,2′:2,1′-β-D-Fru*f* **(20)**	103.4	100.1	83.0	78.7	84.4	77.8	75.4	80.9	63.1 [b]	63.7	62.6 [b]	63.2

[a] Not found in inulin caramel.
[b] These two assignments might be interchanged.

therefore a new compound and the former a previously known compound with a newly-assigned anomeric configuration.

Compounds **9**, **14** and **16** were all revealed upon methylation analysis to contain both 1,2-linked fructofuranose and 1,2-linked fructopyranose residues. The ^{13}C NMR spectrum of **16** was identical with a previously published spectrum [6]. Compound **16** was accordingly assigned the identity β-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride (diheterolevulosan III). Since **12** and **16** have already been assigned as having α,β and β,β configurations respectively, two possibilities remain for the identities of **9** and **14**. They might have both residues with the α configuration or have the furanose residue β- and the pyranose residue α-linked. The ^1H NMR spectra of **9** and **14** gave values of 5.2 and of ~4 Hz for $J_{3',4'}$, respectively. This is indicative of a $^5C_2$ conformation for the fructopyranose ring and this is consistent with an α configuration for the pyranose ring which would be expected to have O-2′ axial in accordance with the anomeric effect. The anomeric and *exo*-anomeric effects have been shown to be the dominant influence upon conformation in dihexulose dianhydrides [9]. Comparison of the ^{13}C and ^1H spectra of **9** and **14** with those of other di-D-fructose dianhydrides [10] led to the assignment of **9** as β-D-fructofuranose α-D-fructopyranose 1,2′:2,1′-dianhydride and **14** as α-D-fructofuranose α-D-fructopyranose 1,2′:2,1′-dianhydride, both being new compounds.

Only trace amounts of compounds **5** and **6** could be isolated, several successive LC runs being required to effect their isolation. Methylation analyses of both revealed 2,3-linked fructofuranose and 1,2-linked fructopyranose. The ^{13}C NMR spectrum of **6** was exactly coincident with the previously published spectrum of β-D-fructofuranose β-D-fructopyranose 2,1′:3,1′-dianhydride [11] isolated from the products of the reaction of anhydrous HF upon inulin and upon fructose. The ^{13}C NMR spectrum of **5** was consistent with β-D-fructofuranose α-D-fructopyranose 2,1′:3,2′-dianhydride and strong cross peaks were observed in the HMBC spectrum between C-2′ and H-3 and between C-2 and H-1′a and H-1′b. This molecule would be expected to have the α-fructopyranose residue in the $^5C_2$ conformation, so that O-2′ would be axially disposed, and the central dioxane ring would be in the boat conformation; both these conformations are dictated by the anomeric and *exo*-anomeric effects [9]. This would have the effect of bringing H-4 into close proximity through-space with O-5′ resulting in deshielding and an accompanying downfield shift of the signal for H-4 as observed in the ^1H spectrum. A similar effect was observed in the ^1H spectrum of **4** [2], which has a β-D-fructofuranose residue linked at O-2 and O-3 to an α-D-fructofuranose residue.

The ^1H NMR and APT spectra of **11** revealed the presence of a glucose as well as a fructose residue. Methylation analysis gave only a single peak corresponding to a 1,2-linked residue. It should however be noted that neither gas chromatography nor mass spectroscopy can distinguish the partially methylated glucitol acetates arising from 1,2-linked glucopyranose and 1,2-linked fructofuranose residues even when borodeuteride is used for the reduction step. If **11** is formed by the condensation of a glucose and fructose residue then the linkage must be of the type 1,1′:2,2′. β-D-Fructofuranose α-D-glucopyranose 1,1′:2,2′-dianhydride has been made by the action of HF upon 1-*O*-α-D-glucopyranosyl-D-fructose [12]. However, the ^{13}C NMR spectrum of **11** was quite unlike the spectrum of this compound, which leaves three possibilities. Compound

11 might have both residues in the β configuration, or both α, or the fructofuranosyl residue might be α- and the glucopyranosyl residue β-linked. The small value of $J_{1',2'}$ in the ^{1}H NMR spectrum precludes the possibility of a β-linked glucopyranosyl residue. This is because examination of models shows that closure of the 1,2-dioxane ring with a β-linked glucopyranosyl residue locks H-1′ and H-2′ in a *trans* relationship which would be expected to give a much larger coupling constant. Compound **11** must therefore have both residues α-linked and be α-D-fructofuranose α-D-glucopyranose 1,1′:2,2′-dianhydride. In this compound the central dioxane ring is likely to adopt a chair conformation which has O-5 and O-5′ both axially disposed [10]. The glucopyranosyl residue cannot readily adopt a conformation in which O-1′ is truly axial. The values of the ^{1}H coupling constants of the glucopyranosyl residue indicate considerable distortion of the normal $^4C_1$ geometry. In order to keep the bulky hydroxymethyl group equatorial, the glucopyranosyl residue can adopt a twist boat conformation. This would result in the dihedral angle between H-2′ and H-3′ approaching 90° and that between H-4′ and H-5′ approaching 180°. This would agree with the observed coupling constants in the ^{1}H spectrum.

It was not possible to isolate **17**, however it is presumed to be a dianhydride because of its mass spectrum. The spectrum of **17** suggests that it contains either one or two pyranose rings (prominent ion at m/z 204).

The information contained in the mass spectra of the dianhydrides cannot be used reliably as a sole indicator of structure for reasons which will be outlined below. Nevertheless gas chromatography and mass spectroscopy (GCFID, GCMS) have proved to be valuable tools in the identification of the dianhydrides. Table 4 summarizes the retention times and mass spectra of the per-*O*-Me_3Si ethers of all fourteen dianhydrides described in this paper. The molecular ion is m/z 756 and m/z 741 is due to $[M-CH_3]^+$. A prominent ion at m/z 653 arises by loss of the Me_3SiOCH_2 moiety of mass 103. Subsequent eliminations of Me_3SiOH (90) from m/z 741 and 653 yield 563, 651, 561 and 471. Significant ions at m/z 451 and 361 representing $[Glycosyl]^+$ and $[Glycosyl\ via\ SiMe_3OH]^+$ [13], which are found in the spectra of singly-linked disaccharides and may constitute the base peak, are notably reduced in or absent from the spectra of the dianhydrides. The ions m/z 73 and 147 are common to the spectra of most trimethylsilylated glycosides [14]. An increased proportion of the m/z 204 ion is indicative of the presence of a pyranose ring whereas the ion m/z 217 predominates when only furanose rings are present [15]. The absence of a prominent m/z 204 ion does not however preclude the presence of a pyranose ring as the spectrum of **11**, which contains a glucopyranosyl residue, attests. Presumably the involvement of O-2′ of the glucopyranosyl residue in the linkage reduces the possible options for the formation of m/z 204 [16]. Similarly a reduced amount of m/z 217 is seen in the 2 → 3-linked furanose-pyranose dianhydrides, **5** and **6**; presumably involvement of O-3 of the furanose residue reduces the likelihood of formation of this ion [16]. We have previously described possible fragmentation pathways for the production of the ions m/z 509 and 362 [2,17]. When observed in the entire caramel **6** showed increased intensities of the ions m/z 217 and 361 indicating a trace presence of another species in the chromatography peak.

It was not possible to isolate any of the singly-linked disaccharides present in the

Table 4
Partial mass spectra of the per-*O*-Me_3Si ethers of the dimeric dianhydrides observed in inulin and sucrose caramels.

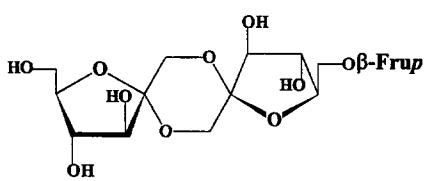
Compound	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
$R_{glucitol}$ [a]	1.710	1.737	1.806	1.862	1.883	1.883	1.905	1.914	1.930	1.953	1.973	1.987	2.022	2.075
m/z	Abundance (%)													
73	100.0	100.0	100.0	100.0	83.0	100.0	100.0	100.0	100.0	67.0	100.0	46.4	89.7	60.8
103	21.5	15.7	17.7	22.5	6.0	10.2	14.6	15.4	10.7	9.6	12.2	7.5	9.2	5.6
147	14.6	20.8	25.4	17.3	23.7	20.4	15.1	21.5	24.1	13.7	20.4	9.4	24.9	19.3
204	0.8	90.5	33.8	1.4	100.0	34.6	1.1	5.3	58.7	0.8	39.5	0.8	55.9	100.0
217	80.5	22.9	19.6	32.8	23.2	60.9	91.4	83.9	74.6	100.0	85.0	100.0	100.0	26.0
361	9.4	13.2	12.2	27.6	2.1	2.0	4.4	11.6	3.9	4.2	4.1	2.2	2.4	3.4
362	3.3	4.8	4.7	9.7	4.2	2.8	2.7	7.6	8.7	6.8	5.4	2.6	4.1	4.1
471	0.3	0.4	0.4	–	0.4	0.5	0.5	0.4	0.6	0.3	0.5	0.2	0.3	–
509	9.3	7.9	1.2	1.5	16.4	20.5	29.2	2.2	23.4	21.4	29.4	12.9	20.1	6.3
561	0.9	0.9	0.5	0.7	0.4	0.5	1.0	1.2	0.5	0.5	0.5	0.4	0.3	–
563	0.6	0.6	–	7.7	–	0.2	0.5	3.5	0.3	0.3	0.2	0.2	–	0.6
651	0.6	0.4	0.7	0.6	0.5	2.7	2.1	1.1	1.5	0.8	0.8	0.4	0.6	–
653	11.2	25.1	32.2	52.1	0.2	0.8	0.8	0.4	0.4	0.3	0.2	–	–	0.6
741	0.1	–	–	–	–	–	0.1	0.4	0.2	–	0.1	–	–	–
756	–	–	–	–	0.3	0.1	–	–	–	–	0.1	–	–	–

[a] Chromatography described in Section 3.

fractions E1 and E2 of sucrose caramel. The mass spectra of the per-*O*-trimethylsilyl ethers were however consistent with the presence of only single inter-residue linkages. Prominent ions were observed at m/z 451 and 361 and in some case m/z 437 was also prominent. This latter ion has been shown to be diagnostic of a 2-linked fructofuranose residue [18]. Upon heating with saturated $Ca(OH)_2$ the singly-linked disaccharides were almost completely consumed indicating that the majority of these disaccharides are reducing sugars. Residual sucrose survived the alkaline treatment. In the GC trace of the per-*O*-Me_3Si ethers, Fig. 3, the singly-linked disaccharides elute more slowly than the majority of the dianhydrides, most of them running more slowly than **13**.

Trimer fraction.—Mild hydrolysis of the SEC trimer fraction (D, Fig. 1b) from sucrose (4.7 μmol) yielded dianhydrides (2.6 μmol), fructose (1.6 μmol) and glucose (1.0 μmol); the remainder of the material was unhydrolyzed. This type of hydrolysis would be expected to cleave singly-linked fructosyl and glucofuranosyl bonds. These results indicate that the trimer fraction contains a significant proportion of dianhydrides with which cations **1**, **2** and **3** have reacted during formation of the caramel. Some of the unhydrolyzed material from the trimers must contain dianhydrides substituted by glucopyranosyl residues but at least part must contain glucotrioses as indicated by the FABMS results. The GC profile of the trimer region of the per-*O*-trimethylsilyl ethers revealed at least 26 peaks indicating a great variety of structures. This profile remained almost unchanged by alkaline degradation confirming that the majority of the trimers are non-reducing. Since no single trimer was outstanding, isolation of individual species was serendipitous. Partial isolation of two trimers, **18** and **19**, was achieved by successive LC runs and these were characterized by NMR spectroscopy. **18** was susceptible to mild hydrolysis. The ^{13}C spectrum of **18** was consistent with **13** substituted at O-6 of the α-D-fructofuranose residue by a β-D-glucofuranose residue. The signal for C-6 of the fructofuranose was downfield shifted by 6.3 ppm and the C-5 signal shifted upfield by 0.8 ppm. Similar effects have been observed in the 6,6′-di-*O*-glucopyranosyl derivative of **13** [19]. In contrast **19** was resistant to mild hydrolysis. The ^{13}C spectrum was consistent with α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride (**13**) substituted at O-6′ of the β-D-fructofuranose residue. The signal for C-6′ was shifted downfield 6.6 ppm and the signal for C-5′ was shifted upfield 1.8 ppm. The coupling constants observed in the ^1H spectrum indicated that the substituent at O-6′ was an α-linked glucopyranosyl residue. Mild hydrolysis would not be expected to cleave this linkage.

In contrast to the sucrose caramel, mild hydrolysis of the trimer fraction from inulin caramel (D, Fig. 1a) resulted in more complete reaction. 10.2 μmol of trimer fraction yielded fructose (8.3 μmol), glucose (0.085 μmol) and dianhydrides (8.9 μmol). Some material remained unhydrolyzed but less than in the sucrose trimer fraction.



20

Trisaccharide **20** was isolated by LC and characterized by NMR spectroscopy and methylation analysis. The spectra were consistent with **13** having been substituted at O-6′ with a β-fructopyranose residue. Substitution with fructose at O-6′ results in an upfield shift of the signal for C-5′ but no concomitant downfield shift of the signal for C-6′ [20]. Methylation analysis confirmed this identification. ^{13}C and ^{1}H spectra of compounds **18–20** are given in Tables 3 and 5.

The presence of O-6 or O-6′ substituted dianhydride **13** in all three of the trisaccharides that have been isolated is not suprising. When mild hydrolyses of the trimer fractions of inulin and sucrose caramels were compared with the dianhydrides originally present in the dimer fraction of the caramel. Compound **13** was disproportionately represented in the hydrolysates. This most likely results from the fact that it is the preponderant dianhydride in the reaction mixture and therefore the one most likely to be substituted. In addition it was seen that dianhydrides containing fructopyranose residues, **5**, **6**, **8**, **9**, **12**, **14**, and **16** were proportionately less generated by the mild hydrolyses of the trimer fractions than were the difuranose dianhydrides, e.g. **4**, **7**, **10**, **13**, and **15**. This indicates that substitution by glycosyl cation at primary positions of the dianhydrides predominates, a not unexpected effect given the bulk of the incoming cations.

3. Experimental

General procedures.—Methylation of dianhydrides was achieved with NaOH and MeI in Me_2SO [21] with modifications described in ref. [22]. Methylated samples were hydrolysed using the moderate hydrolysis procedure described in ref. [23], reduced with $NaBD_4$ and acetylated by the method of Blakeney et al. [24]. Mild hydrolyses of unmethylated caramel fractions were carried out using 1 M HOAc (90 °C, 90 min). Alkaline degradations were carried out by heating with saturated $Ca(OH)_2$ (100 °C, 120 min), filtering while hot, evaporating to dryness (< 40 °C) and per-*O*-trimethylsilylating for GC.

Inulin and sucrose thermolyses.—Dried inulin (Sigma, from *Dahlia* tubers) or finely powdered sucrose was mixed with anhydrous, amorphous citric acid (1.5% w/w), which had been ball-milled previously. Mixing was accomplished by tumbling for 60 min. Random samples were titrated with alkali to ensure even mixing. Samples were heated in air in an oil bath at 150 °C ± 1 °C with constant stirring. At 7 min for inulin, or 5 min in the case of sucrose, the sample melted to a viscous liquid. After melting the sample was retained at 150 °C with stirring for a further 22 min (inulin) or 7 min (sucrose) and then allowed to cool to a dark golden-brown glass.

Gas chromatography.—GC of per-*O*-Me_3Si ethers of the dianhydrides and of partially methylated alditol acetates was carried out using a Hewlett–Packard Ultra 2 (25 × 0.33 mm) cross-linked phenyl methyl silicone fused silica capillary column. The program for the per-*O*-Me_3Si ethers was 55 °C (1 min) + 30 °C/min to 180 °C and 4 °C/min to 320 °C. The program for the partially methylated alditol acetates was 55 °C (1 min) + 30 °C/min to 220 °C and 10 °C/min to 320 °C.

Mass spectrometry.—Samples were introduced via direct interface of a Hewlett–Packard Ultra 2 (25 × 0.2 mm) cross-linked phenyl methyl silicone fused silica capillary

Table 5
^{1}H NMR spectra of trisaccharides isolated from inulin and sucrose caramels

Compound	Proton chemical shift (ppm) Coupling constant (Hz)														
	H-1_a $J_{1a,1b}$	H-1_b	H-3 $J_{3,4}$	H-4 $J_{4,5}$	H-5 $J_{5,6a}$	H-6_a $J_{5,6b}$	H-6_b $J_{6a,6b}$	H-$1'_a$ $J_{1'a,1'b}$	H-1'b	H-3' $J_{3',4'}$	H-4' $J_{4',5'}$	H-5' $J_{5',6'a}$	H-$6'_a$ $J_{5',6'b}$	H-$6'_b$ $J_{6'a,6'b}$
								H-1 $J_{1,2}$	H-2 $J_{2,3}$	H-3 $J_{3,4}$	H-4 $J_{4,5}$	H-5 $J_{5,6a}$	H-6_a $J_{5,6b}$	H-6_b [a] $J_{6a,6b}$
6-*O*-β-D-Glc*f*-α-D-Fru*f*-1,2′:2,1′-β-D-Fru*f* (**18**)	4.19 d 12.4	3.65 d	3.98 d 2.0	3.95 dd 6.0	4.11 m 2.8	3.93 dd 6.0	3.66 dd 12.8	4.10 d 12.4	3.57 d	3.83 d 8.4	4.06 d ~ 0	3.88 dd	3.74 dd 3.6	3.65 dd 12.4
								5.02 s	4.19 d 0.8	4.21 bd 5.6	4.16 dd 1.2	~ 3.97 m 3.2	3.84 dd	3.66 dd 12.4
6′-*O*-α-D-Glc*p*-α-D-Fru*f*-1,2′:2,1′-β-D-Fru*f* (**19**)	4.14 d 12.4	3.67 d	3.99 d 2.8	3.91 dd 6.0	3.97 m 2.0	3.74 dd	~ 3.68 12.0	4.13 d 12.0	3.58 d	3.85 d 8.0	4.08 dd 2.8	~ 4.04 m	~ 3.6	
								4.94 d 3.6	3.54 dd 9.8	~ 3.72 9.6	3.40 dd 9.2	~ 3.68	3.7–3.88	
								H-1_a	H-1_b	H-3	H-4	H-5	H-6_aH-6_b [b]	
6′-*O*-β-D-Fru*p*-α-D-Fru*f*-1,2′:2,1′-β-D-Fru*f* (**20**)	4.11 d 12.4	3.68d	3.99 d 2.8	3.91 dd 6.0	3.97 m	~ 3.84	~ 3.70	4.12 d 12.0	3.58 d	3.84 d 8.0	4.41 dd 7.8	3.99 m	~ 3.84	~ 3.70
									3.79	3.88	3.88	3.99	~ 3.70	~ 3.80

[a] Assignments for glucosyl residues.
[b] Assignments for fructopyranosyl residue.

column using the temperature programs described above. Mass spectra were obtained with a Hewlett–Packard 5970 mass spectrometer (70 eV). FABMS and electrospray-MS of underivatized samples and high resolution MS of per-*O*-methylated samples were carried out by J. Sears of the Mass Spectrometry Facility of Montana State University.

The FAB analyses were performed on a VG 70E-HF double focusing magnetic mass spectrometer. The fast atom gun was operated at 8 keV and 1 mA of current using xenon as the gas source. The mass spectrometer was operated at a resolution of 1500 and scanned exponentially down from mass 1500–150 at a rate of 10 s per mass decade. The mass spectrometer was calibrated using glycerol as the calibration compound (mass markers are generated every 92 amu out to 1500 amu). Approximately 2 mL of the sample, dissolved in water, was mixed on a stainless-steel target with 1 mL of glycerol and 1 mL of dithioerythritol and dithiothreitol (5:3). The water in the mixture was allowed to pump away in the FAB probe interface prior to introduction to the high vacuum. Data was acquired in the multichannel averaging mode where 10 scans were summed to generate a single mass spectrum. The signal was acquired and processed using a VG SIOS interface and VG OPUS software running on a DEC Alpha computer.

The electrospray analyses were performed on a VG TRIO-2 quadrupole mass spectrometer equipped with a home-built electrospray ion source. The mass spectrometer was scanned from mass 100–1500 at 10 seconds per scan. Samples were diluted into a solvent consisting of 50:50:2 methanol–water–acetic acid (50:50:2) containing 10^{-3} molar sodium ion. The sample was infused at a rate of 0.50 μl/min using a Harvard apparatus syringe pump (model 22-5555). The mass spectrometer mass scale was calibrated using a polyethylene glycol mixture which provided mass markers every 44 amu throughout the mass range. Ten scans were summed in the multi-channel averaging mode to generate a single mass spectrum of the sample as it was infused to the electrospray needle. Data was acquired and processed using the VG transputer interface and MassLynx software operating on a DEC pentium PC.

Liquid chromatography.—Initial LC separations, which typically yielded simplified mixtures of 2–4 dimers or trimers, were obtained with three Waters Delta-Pak C_{18} 25 × 100 mm Radial-Pak cartridges in series eluted with water at 12 mL/min. Samples were further resolved by repetition of this reverse phase chromatography and by using a Phenomenex RSO-oligosaccharide 200 × 10 mm column eluted with water at 0.33 mL/min at 75 °C. This latter column permitted the separation of dimers and trimers.

Size-exclusion chromatography.—SEC was carried out using a 2.5 × 95 cm column of Bio-Gel P-2 eluted with water at 0.6 mL/min. For preparative purposes 100 mg samples were loaded.

NMR spectrometry.—NMR spectra were obtained using a Varian Unity *plus* 400 MHz spectrometer in D_2O and referenced to internal *t*-BuOH having a ^1H chemical shift of 1.203 ppm and a ^{13}C chemical of 30.695 ppm relative to an external standard of Me_4Si. ^1H,^{13}C, H,H-COSY, APT, HMQC and HMBC spectra were obtained using standard software applications packages. For HMQC a *J* value of 140 Hz was used and for HMBC a delay value of 50 ms was used for evolution of long-range couplings.

Characterization of dianhydrides.—Compounds **4**, **7**, **12** and **13** have been isolated previously from sucrose [1] and inulin [2] caramels.

β-D-Fructofuranose α-D-fructopyranose 2,1′:3,2′-dianhydride **5**.—This compound

was isolated as a colorless glass, $[\alpha]_D^{25}$ −180° (*c* 0.5, H_2O); found (for per-*O*-methylated **5**) $[M + H]^+$: 409.2056, calculated for $C_{18}H_{33}O_{10}$: 409.2074. Methylation analysis yielded 1,2,6-tri-*O*-acetyl-(2-deuterio)-3,4,5-tri-*O*-methylhexitol and 2,3,5-tri-*O*-acetyl-(2-deuterio)-1,4,6-tri-*O*-methylhexitol. The latter was present in reduced amount relative to the former, which effect was also observed during methylation analyses of **4** and **7** [2].

β-D-*Fructofuranose β*-D-*fructopyranose 2,1′:3,2′-dianhydride* **6**.—This compound was isolated as a colorless glass, $[\alpha]_D^{25}$ −68° (*c* 0.44, H_2O); lit. −58.5° (20°, *c* 1.03, H_2O) [11]. The disparity between the observed and literature values of optical rotation is probably due to the small amounts of material available, only a few milligrams were isolated after several successive LC runs. Methylation analysis yielded 1,2,6-tri-*O*-acetyl-(2-deuterio)-3,4,5-tri-*O*-methylhexitol and 2,3,5-tri-*O*-acetyl-(2-deuterio)-1,4,6-tri-*O*-methylhexitol. This was the most intractable of the per-*O*-methylated dianhydrides with respect to hydrolysis; eventually the concentration of CF_3CO_2H was increased from 1.5 to 3% and the temperature raised to 125 °C; these conditions resulted in a partial hydrolysis. We have previously noted a resistance to hydrolysis related to the 2,3-linked fructofuranose residue [2]. The relative amounts of the two partially methylated alditol acetates were similar to those observed with **5**.

α-D-*Fructopyranose β*-D-*fructopyranose 1,2′:2,1′-dianhydride* **8**.—This compound could not be isolated in sufficiently pure form for measurement of optical rotation or accurate methylation analysis. However, the per-*O*-Me_3Si ether of **8** co-eluted with that of an authentic sample and had the same mass spectrum. Moreover the ^{13}C NMR spectrum of the mixture containing **8** contained peaks corresponding to all of those of the authentic compound [6].

β-D-*Fructofuranose α*-D-*fructopyranose 1,2′:2,1′-dianhydride* **9**.—This compound was obtained as a colorless glass, $[\alpha]_D^{25}$ +4.8° (*c* 2.1, H_2O); found (for per-*O*-methylated **9**) $[M + H]^+$: 409.2073, calculated for $C_{18}H_{33}O_{10}$: 409.2074. Methylation analysis yielded approximately equal amounts of 1,2,5-tri-*O*-acetyl-(2-deuterio)-3,4,6-tri-*O*-methylhexitol and 1,2,6-tri-*O*-acetyl-(2-deuterio)-3,4,5-tri-*O*-methylhexitol.

Di-α-D-*fructofuranose 1,2′:2,1′-dianhydride* **10** *and di-β*-D-*fructofuranose 1,2′:2,1′-dianhydride* **15**.—Both **10** and **15** were obtained as colorless glasses. Methylation analyses yielded only 1,2,5-tri-*O*-acetyl-(2-deuterio)-3,4,6-tri-*O*-methylhexitol. The ^{13}C NMR spectrum of **10** was identical with the previously-published spectrum of a compound tentatively assigned as having both of the fructofuranose residues in the *β* configuration [7]. However comparison of the NMR spectra of **10** and **15** led to the assignment of **10** as *di α*-D-*fructofuranose 1,2′:2,1′-dianhydride*, $[\alpha]_D^{25}$ +114.8° (*c* 6.0, H_2O); lit. [7] +93° (H_2O); found (for per-*O*-methylated **10**) $[M + H]^+$: 409.2081, calculated for $C_{18}H_{33}O_{10}$: 409.2074. **15** was assigned as *di-β*-D-*fructofuranose 1,2′:2,1′-dianhydride*, $[\alpha]_D^{25}$ −53.7°(*c* 5.4, H_2O); found (for per-*O*-methylated **15**) $[M + H]^+$: 409.2072 calculated for $C_{18}H_{33}O_{10}$: 409.2074.

α-D-*Fructofuranose α*-D-*glucopyranose 1,1′:2,2′-dianhydride* **11**.—This compound was isolated as a colorless glass, $[\alpha]_D^{25}$ +26.8° (*c* 2.98, H_2O); found (for per-*O*-methylated **11**) $[M + H]^+$: 409.2075, calculated for $C_{18}H_{33}O_{10}$: 409.2074. Methylation analysis yielded only 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylhexitol, the position of deuteration could not be distinguished. In the HMBC spectrum cross peaks were observed relating

C-1 of glucose with H-1 of fructose and C-1 of fructose with H-1 of glucose. In the ^{1}H spectrum the signals for H-1, H-2, H-3 and H-4 of glucopyranose were downfield shifted compared with methyl glucopyranoside whereas the signals for H-6a and H-6b were almost unchanged. This indicates conformational strain in the pyranose ring. In the ^{13}C spectrum the signals for C-2 and C-3 of glucose were downfield shifted by about 1 ppm whereas the signal for C-4 was shifted downfield by 11 ppm indicating extreme distortion and compression such as might occur in a twist boat conformation.

α-D-Fructofuranose α-D-fructopyranose 1,2′:2,1′-dianhydride **14**.—Compound **14** was isolated as a colorless glass, $[\alpha]_D^{25}$ +94.1° (*c* 1.6, H_2O); found (for per-*O*-methylated **14**) $[M + H]^+$: 409.2088, calculated for $C_{18}H_{32}O_{10}$: 409.2074. Methylation analysis yielded approximately equal amounts of 1,2,5-tri-*O*-acetyl-(2-deuterio)-3,4,6-tri-*O*-methylhexitol and 1,2,6-tri-*O*-acetyl-(2-deuterio)-3,4,5-tri-*O*-methylhexitol.

β-D-Fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride **16**.—This compound was isolated as a colorless glass. Methylation analysis yielded approximately equal amounts of 1,2,5-tri-*O*-acetyl-(2-deuterio)-3,4,6-tri-*O*-methylhexitol and 1,2,6-tri-*O*-acetyl-(2-deuterio)-3,4,5-tri-*O*-methylhexitol. The ^{13}C NMR spectrum was identical with one previously published for this compound [6]. The optical rotation was −189° (*c* 1.3, H_2O); lit. −179° (H_2O) [25], −183° (H_2O) [26].

Isolation and characterization of trisaccharides.—Compounds **18** and **19** could not be isolated in sufficiently pure form to afford an accurate optical rotation. Structures were assigned by NMR spectroscopy and comparison with literature [27]. The NMR spectra of **18** were consistent with *6-O-(β-D-glucofuranosyl)-α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride*, whilst those of **19** were consistent with *6′-O-(α-D-glucopyranosyl)-α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride*.

6′-O-(β-D-Fructopyranosyl)-α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride **20**.—This compound was isolated as a colorless glass, $[\alpha]_D^{25}$ −10.0° (*c* 3.0, H_2O); found (for per-*O*-methylated **20**) $[M + NH_4]^+$: 630.3333, calculated for $C_{27}H_{52}N_1O_{15}$: 630.3337. Methylation analysis yielded 2,6-di-*O*-acetyl-(2-deuterio)-1,3,4,5-tetra-*O*-methylhexitol, 1,2,5-tri-*O*-acetyl-(2-deuterio)-3,4,6-tri-*O*-methylhexitol and 1,2,5,6-tetra-*O*-acetyl-(2-deuterio)-3,4-di-*O*-methylhexitol in approximately equal amounts.

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