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Formation of trisaccharides (kestoses) by pyrolysis of sucrose

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ABSTRACT

Amorphous sucrose, containing citric acid as catalyst, undergoes thermolysis at 100° to yield fructofuranosyl cation and D-glucose. The cation reacts with unchanged sucrose to form all three of the known kestoses, and also their α-fructofuranosyl anomers. Two of the latter are resistant to invertase hydrolysis. A new fructosylglucose disaccharide is also formed.

INTRODUCTION

Previous studies on the thermal degradation of sucrose[1,2] have indicated the formation of trisaccharides, presumed to be kestoses (fructosylsucrose derivatives). The thermal decomposition of sucrose proceeds[3] via scission of the glycosidic linkage to form a resonance-stabilized fructosyl cation which may then be subject to nucleophilic attack by oxygenatoms of alcohols present in the reaction mixture. Such transfructosylations have been studied in detail with simple alcohols[3]. The assumption of kestose formation just mentioned was predicated upon the fact that the hydroxyl groups of untreated sucrose could also act as nucleophiles. The primary hydroxyl groups are the most likely to act thus, since the earlier studies[3] showed that the fructosyl cation reacts more readily with primary than with secondary alcohols.

Bollmann and Schmidt-Berg[4] had earlier heated dry sucrose at 170° and identified, by paper chromatography, thirteen products, of which five were nonreducing trisaccharides; one of these being unequivocally identified as 6-kestose. Most of the literature references to kestoses pertain to their biological origins. These trisaccharides, together with their higher d.p. homologues, are found in a number of flowering plant families[5], in which they constitute precursors of the fructan storage-polymers and have been implicated in frosthardiness and osmotic control[6,7].

There has also been some recent interest[8] in the enzymic preparation from sucrose of 1-kestose and its higher homologues, which are reported to have potential as nonnutritive sweeteners[8–10]. We now report a detailed examination of the products of acid-catalyzed thermal degradation of sucrose.

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RESULTS AND DISCUSSION

The products of sucrose thermolysis were expected to include kestoses containing fructose moieties linked to sucrose at the three primary hydroxyl positions. Application of conventional methylation analysis (sequential methylation, hydrolysis, reduction, and acetylation) did not permit the g.l.c. separation of the trimethyl ether derivatives resulting from the 1'- and 6'-fructosyl derivatives, although borodeuteride reduction would differentiate the mass spectra (see ref. 11). Therefore, we sought a more discriminating method of analysis. It was known that partially methylated and subsequently acetylated aldoses[12] and ketoses[13] can be well resolved on g.l.c. despite the problems of anomerization, and that they give mass spectra which are readily distinguishable. We

TABLE I

Reference compounds for methylation analysis

Compound	*Source*
2,3,4,6-Tetra-*O*-methyl-D-glucose acetate (**1**)	methyl α-D-glucopyranoside
1,3,4,6-Tetra-*O*-methyl-D-fructose acetate (**2**)	methyl β-D-fructofuranoside
2,3,4-Tri-*O*-methyl-D-glucose diacetate (**3**)	dextran
2,3,6-Tri-*O*-methyl-D-glucose diacetate (**4**)	amylose
1,3,4-Tri-*O*-methyl-D-fructose diacetate (**5**)	6'-*O*-tritylsucrose[25]
3,4,6-Tri-*O*-methyl-D-fructose diacetate (**6**)	1-kestose
6-*O*-Acetyl-1,2,3,4,5-penta-*O*-methyl-D-glucitol (**7**)	isomaltitol

TABLE II

Gas chromatography of partially methylated acetylated sugars

Compound[a]		*Approximate relative peak areas*	*Relative retention time (R_T)*
2	Peak 1	1	1.00
	Peak 2	15	1.09
1	Peak 1	2	1.08
	Peak 2	1	1.22
3	Peak 1	1	2.61
	Peak 2	1	2.82
4	Peak 1	6	3.29
	Peak 2	1	3.57
5[b]			2.79
6[b]			3.19
7[b]			1.21

[a] Numbered as Table I. [b] Only one g.l.c. peak.

TABLE III

Partial mass spectra of reference compounds for methylation analysis

Reference compound[a] (m/z)	1	2	3	4	5	6
69	1	5	3	4	55	3
71	10	24	7	21	86	12
73	6	10	8	12	17	12
75	8	10	16	14	25	6
87	6	22	14	100	54	24
88	23	7	25	40	21	6
89	5	47	3	3	26	16
99	2	8	4	18	71	6
101	100	100	100	25	100	100
102	6	5	4	2	7	6
103	1		1	5	43	3
113	1	2	3	12	10	2
115	6	12	8	10	10	3
127	3	5	6	25	13	6
129	2		8	80	5	2
145	1	20	1	2	1	9
146		2				2
159	1	20		5	17	1
187	5			5	Tr	18
191		22			1	1
218		3				
219				1	25	
233				4		
246					3	
247			3	3		

[a] Numbered as Table I.

have therefore applied this type of approach (namely methylation, hydrolysis, acetylation) to structural analysis of the kestoses. The products were identified by g.l.c.–m.s. comparison with authentic standards, the origins of which are indicated in Table I. G.l.c. retention times and partial mass spectra of these standards are shown respectively in Tables II and III. The fragmentation patterns of **1** and **4** have already been discussed[14] and the mass spectrum of **3** is readily explainable in the context of previously published material[14,15].

The mass spectra of the fructofuranoside derivatives have not previously been discussed directly, but peaks at *m/z* 159, 101, and 129 in compounds **2** and **5** and *m/z* 187 and 129 in compound **6** are consistent with a fragmentation pattern proposed by Karady and Pines[16] for trimethylsilyl ethers of ketofuranosides. Kochetkov and Chizov[17] have proposed fragmentation pathways for methyl furanosides which could account for the formation of ions 103, 88, 101, and also 146 and 115 from **2** and **6** and the corresponding ions 174 (not observed), 143, and 115 from **5**. The propensity of furanoside rings to undergo sequential elimination rather than major fragmentation of the ring has been noted previously[16–19], and in accordance with this, peaks at *m/z* 191,

145, and 113 in **2** and **6** are consistent with successive eliminations of C-1 with its substituent, the acetoxyl group of C-2 as ketene, C-6 with its substituent and methanol. The corresponding peaks in **5** are at *m/z* 219, 145, and 113. The foregoing type of methylation analysis has now been used to determine the structure of the trisaccharides formed in thermolysis of sucrose.

When pure crystalline sucrose (**8**) is heated it remains relatively stable until melting at ~187°, whereupon the disappearance of sucrose occurs rapidly; thus 88% has reacted in 12 min at 190° (ref. 2). The thermolysis of pure sucrose can be observed at much lower temperatures if it is not crystalline[1]. Hence, we have used amorphous sucrose in the present study and have generated this product by rapid evaporation at room temperature of a concentrated aqueous solution. The thermolysis has been conclusively shown to be acid catalyzed in methyl sulfoxide solution[20] and hence we have included a small amount of acid catalyst in the preparation of the amorphous sucrose, thereby ensuring also a uniform distribution of the catalyst in the acid–sucrose melts. The choice of acid catalyst was influenced by the high level of acid sensitivity of the sucrose glycosidic linkage and by the requirement that the catalyst be nonvolatile at likely reaction temperatures (such as 100–160°). Several carboxylic acids of low volatility were assayed and citric acid was selected for the detailed studies. This acid gave products which were much less colored than some alternative catalysts (for instance tartaric or phosphoric acids)[21], for reasons which are not known with certainty, but which may involve interception of free-radical intermediates in color (caramel) formation by the tertiary C-H group.

Thermal degradation of the acid–sucrose melts was carried out under vacuum in a heated oil-bath. The vacuum probably does not significantly influence the thermolysis reactions, but it ensures the rapid removal of the last traces of water during heat-up to reaction temperature. Obviously, any water remaining in the melt would very rapidly be incorporated by hydrolysis, with attendant loss of sucrose. The first step in the acid-catalyzed thermolysis of sucrose is the formation of the resonance-stabilized fructofuranosyl cation and glucose[20], and in our system the former then reacts relatively efficiently with the oxygen nucleophiles of the primary alcohol groups of unreacted sucrose to generate kestoses. It was anticipated that the fructosyl cation would react with equal facility with the primary hydroxyl group of product glucose.

The products of heating the acid–sucrose melts (100°/60 min) were subjected to a preliminary fractional precipitation, which separated much of the glucose and some of the unreacted sucrose. The precipitated oligosaccharide products were then fractionated by two successive l.c. procedures, namely system (*a*) and then (*b*). The l.c. profiles given by the total precipitated products in each system are shown in Fig. 1 and 2.

The four major products, isolated by this procedure, included two known compounds, namely 6-kestose (**11**) and neokestose (**13**). These were identified by l.c. comparison with authentic compounds and the structures confirmed by methylation, hydrolysis, acetylation, and g.l.c.–m.s. (Table IV). The third pure compound thus isolated proved to be an α-fructosylsucrose anomer of 6-kestose which was accorded the trivial name of iso-6-kestose (**12**, Tables IV and V). The remaining major product

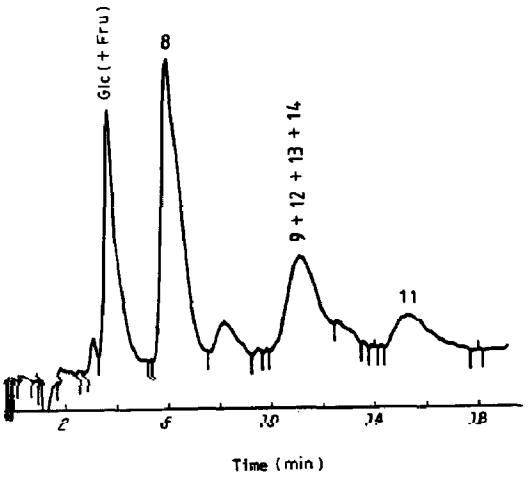


Fig. 1. Products from acid–sucrose melt (100°/60 min); l.c. system (*a*).

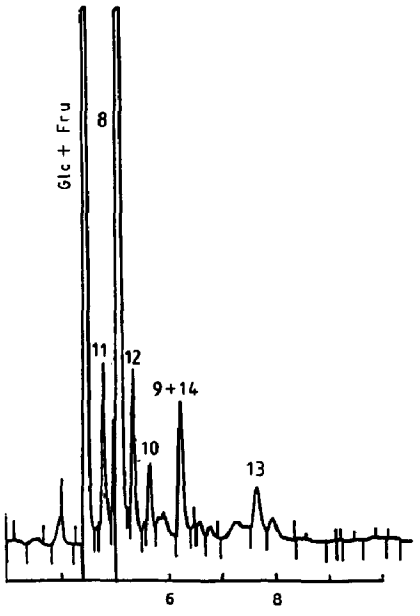
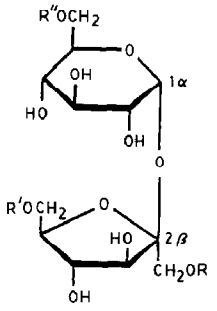


Fig. 2. Products from acid-sucrose melt (100°/60 min); l.c. system (*b*).

isolated in this way ran concurrently with 1-kestose on both l.c. systems; however methylation analysis revealed, in addition to the expected products derived from 1-kestose (**9**), a larger amount of products which would be expected to arise from neokestose (**13**). This was confirmed by ^{13}C-n.m.r. spectroscopy (Table VI), which indicated a preponderance of iso-neokestose (**14**, the α-fructosylsucrose anomer of neokestose) with some 1-kestose (**9**). A second work-up procedure involving pretreat-



8 Sucrose, R = R′ = R′′ = H.

9 1-Kestose, R = β-D-fructofuranosyl, R′ = R′′ = H.

10 Iso-1-kestose, R = α-D-fructofuranosyl, R′ = R′′ = H.

11 6-Kestose, R′ = β-D-fructofuranosyl, R = R′′ = H.

12 Iso-6-kestose, R′ = α-D-fructofuranosyl, R = R′′ = H.

13 Neokestose, R′′ = β-D-fructofuranosyl, R = R′ = H.

14 Iso-neokestose, R′′ = α-D-fructofuranosyl, R = R′′ = H.

ment with invertase before precipitation (Fig. 3) yielded three products amenable to isolation by l.c. (system *b*). One of these was iso-6-kestose (**12**). The second was a reducing disaccharide containing fructose and glucose. ^{1}H- and ^{13}C-n.m.r. spectroscopy and borodeuteride reduction followed by methylation analysis indicated that this product was 6-*O*-α-D-fructofuranosyl-D-glucopyranose (Tables IV and V). A small amount of this compound was detected by l.c. in the original mixture (Fig. 2), but it was greatly augmented by the enzyme treatment, doubtless because of the partial hydrolysis of iso-neokestose (**14**). The presence of this compound among the products of sucrose thermolysis at 170° had previously been tentatively deduced[4]. The final product obtained from the invertase pretreatment was iso-1-kestose (**10**, the α-fructosylsucrose anomer of 1-kestose, Tables IV and V).

When pure samples of the kestose products became available it was possible to determine their l.c. response factors. The progressive contents of four of the kestose products in acid–sucrose melts heated at 100° for various times were then determined by l.c. (method *b*) and are shown in Fig. 4. Iso-6-kestose (**12**) forms rapidly to reach a maximum at ~1 h and then decreases, presumably by undergoing thermolysis to form either a fructosyl cation and sucrose or a difructosyl cation and glucose. 6-Kestose (**11**) is formed at a similar rate, but is less rapidly degraded, perhaps suggesting that the α-fructofuranosyl linkage undergoes thermolysis more rapidly than the comparable β-fructofuranosyl linkage. Unfortunately, it was not possible to confirm this by comparison of the other two isomeric pairs because iso-neokestose (**14**) and 1-kestose (**9**) migrated concurrently under the l.c. system used. The combined curve for these two is not shown in Fig. 4, but was similar in form and magnitude to that for iso-6-kestose (**12**).

TABLE IV

Methylation analysis of products of sucrose thermolysis

Compound	*Products of methylation analysis*[a]
6-Kestose (**11**)	**1, 2, 5**
Iso-6-kestose (**12**)	**1, 2, 5**
Neokestose (**13**)	**2, 3**
Iso-1-kestose (**10**)	**1, 2, 6**
1-Kestose, iso-neokestose mixture (**9** + **14**)	**1, 2, 3, 6**
6-*O*-α-Fructofuranosyl-D-glucose	**2, 7** (1-^{2}H)

[a] Numbered as Table I.

TABLE V

Assignments of ^{13}C-n.m.r. spectra of new oligosaccharides in D_2O[a]

Carbon[b] *atom*	*1-Kestose*[c] (**9**)	*Iso-1-kestose*[a,e] (**10**)	*6-Kestose*[d,e] (**11**)	*Iso-6-kestose*[d,e] (**12**)	*6-α-Fructosyl-D-glucose*[d,f]	
					β[g]	α
1′	62.17	62.2	62.2	62.2		
1″	61.70	59.9	61.4	59.4	59.3	59.3
2′	104.50	104.3	104.8	104.6		
2″	104.96	109.1	104.8	109.4	109.2	109.0
3′	77.92	77.2	77.2	77.0		
3″	77.94	81.9	77.2	81.2	81.1	81.3
4′	75.12	74.6	75.8	75.2		
4″	75.74	78.0	75.4	78.2	78.2	78.2
5′	82.46	82.3	82.5	80.6		
5″	82.36	84.1	82.5	84.3	84.2	84.0
6′	63.44	63.1	70.2	63.4		
6″	63.59	61.6	63.6	62.2	61.0	61.0
1	93.73	93.3	93.2	93.0	96.8	93.0
2	72.39	72.0	72.0	72.0	74.9	72.2
3	73.85	73.6	73.6	73.6	76.6	73.6
4	70.48	70.2	70.6	70.3	70.6	70.6
5	73.67	73.3	73.4	73.2	75.3	71.0
6	61.40	61.1	60.8	61.4	62.0	62.0

[a] Relative to internal 1,4-dioxane at 67.4 p.p.m. [b] Double primed numbers refer to the fructose unit appended to sucrose in the kestoses and to glucose in the disaccharide. [c] From ref. 29. [d] Based on related compounds assigned in refs. 26–29. [e] Measured at 22.5 MHz. [f] Measured at 90 MHz. [g] Dominant anomer.

The lower yield of iso-1-kestose (**10**) is reminiscent of the general lower reactivity of the 1′-hydroxyl group of sucrose in selective derivatization[22].

The relative reactivities of the kestoses to invertase were determined with yeast invertase (*β*-D-fructofuranosidase) and are shown in Table VII in comparison with sucrose. Iso-neokestose is not included because it was not available in pure form. The presence of an α-D-fructofuranosyl group at either of the primary positions of the fructose residue would be expected to confer complete immunity to attack by a

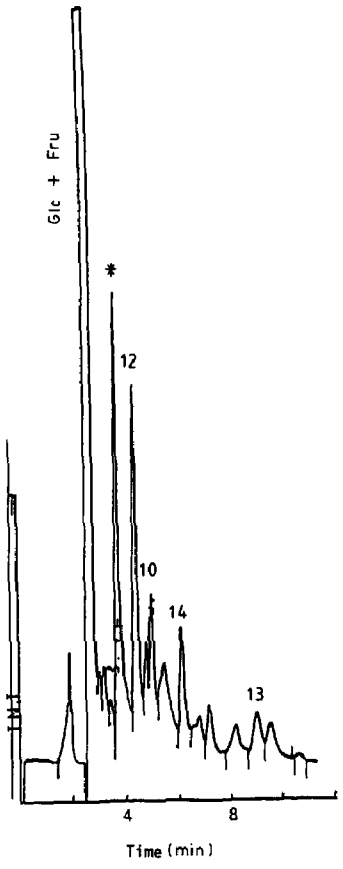


Fig. 3. L.c. of product mixture after invertase treatment (system *b*) [* = 6-*O*-α-D-fructofuranosyl-D-glucose].

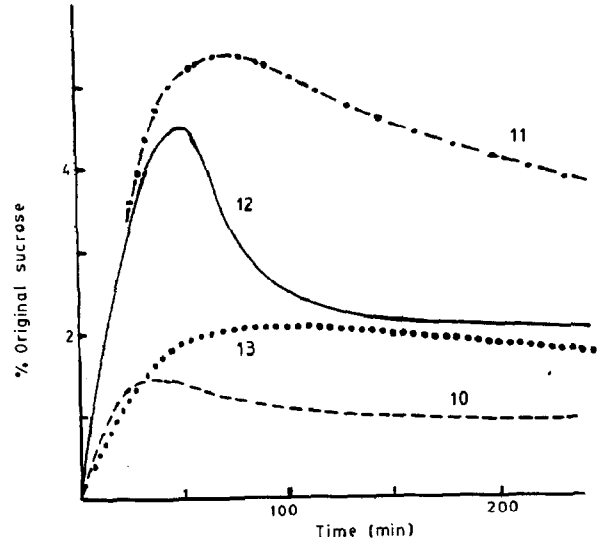


Fig. 4. Yields of kestoses from 1% citric acid–sucrose melt at 100°.

TABLE VI

Chemical shifts observed in the spectra of a mixture of iso-neokestose (**14**) and 1-kestose (**9**) in D_2O with tentative assignments[a]

Chemical shift[b]	*1-Kestose*[c]	*Iso-neokestose*[c] (**14**)
109.3		2″
105.0	2″	2′
104.5	2′	
93.8	1	
93.4		1
83.9		5″
82.6, 82.5, 82.5, 82.0	5′, 5″	5′, 3″
78.2		4″
77.9, 77.7	3′, 3″	3′
75.8	4″	
75.4, 75.1	4′	4′
73.9	3	3
73.7	5	
72.6		2
72.4	2	
72.3		5
70.8		4
70.5	4	
63.9, 63.6, 63.5	6′, 6″	6′
62.6		6
62.4, 62.2	1′	1′
61.7	1″	
61.4	6	
61.2		6″
60.3		1″

[a] Based on related compounds assigned in refs. 26–29. [b] Measured at 90 MHz relative to internal 1,4-dioxane at 67.4 p.p.m. [c] As for footnote[b] in Table V.

TABLE VII

Action of invertase (β-D-fructofuranosidase) on kestoses

Substrate	$t_{0.5}$ (*min*)[a]
Sucrose (**8**)	$<<0.5$
1-Kestose (**9**)	3.5
6-Kestose (**11**)	1.2
Neokestose (**13**)	3.8
Iso-1-kestose (**10**)	No reaction detected up to 24 h.
Iso-6-kestose (**12**)	No reaction detected up to 20 h.

[a] Time, in min, taken for loss of 50% of starting material.

β-fructofuranosidase, and this is borne out by the isolation of unchanged iso-1-kestose and iso-6-kestose after drastic invertase pretreatment of the oligosaccharide products from sucrose thermolysis. Isoneokestose, on the other hand, has an exposed β-fructofuranosyl residue and it is tentatively concluded that, when the oligosaccharide mixture was subjected to enzyme treatment, isoneokestose was degraded to 6-*O*-α-fructosylglucose (see earlier and Fig. 3).

EXPERIMENTAL

General. — L.c. was performed with either (*a*) a Waters μBondapak NH_2, 8 mm × 10 cm, 10 μm, Radial-Pak cartridge column with 3:2 MeCN–water at 4 mL/min; or (*b*) a Waters Resolve C_{18}, 8 mm × 10 cm, 5 μm Radial-Pak cartridge column with water at 1 mL/min and a Waters differential refractometer R401. Separation of the product mixture into its components was achieved by successive preparative l.c. through (*a*) and then (*b*) after the method of Ivin and Clarke[23]. Optical rotations and some n.m.r. spectra were measured by T. Lowary at the University of Edmonton. Molecular weights and formulas were determined by f.a.b.–m.s. carried out by J. Sears at the Mass Spectrometry Facility of Montana State University, using xenon atoms at 8 KeV and 1 mA current, with a glycerol matrix, polyethylene glycol 400 reference, and a mass resolution of 5000. Authentic kestoses were a gift from M. A. Clarke and W. S. C. Tsang of Sugar Processing Research, Incorporated, New Orleans, LA.

Methylation analysis of oligosaccharide products. — Methylations were performed according to Ciucanu and Kerek[24]. Samples (0.5–2 mg) were dissolved in Me_2SO (0.5 mL) with stirring in a capped vial, the solution was cooled to ~10° and finely powdered NaOH (20–30 mg) added, followed after 10–15 min by MeI (200 μL). The resulting mixture was stirred for 45 min at 10°, and then the excess of MeI was removed in a stream of dry air. Water (1.0 mL) and chloroform (1.0 mL) were added and mixed and the upper layer was discarded. The $CHCl_3$ layer was washed with water (3 × 1.0 mL) and finally evaporated under a stream of dry air.

Methylated fructofuranosides and kestoses were hydrolyzed with 90% HOAc (100 μL) for 10 min at 100° in a sealed vial followed by the addition of water (500 μL) and further heating for 60 min at 100°. In the case of methylated glucopyranosides, the HOAc was replaced with CF_3CO_2H. Acetylation of dried hydrolysis products was carried out in dry pyridine (100 μL) and Ac_2O (100 μL) overnight at 25°, prior to g.l.c.–m.s., which was carried out with a Hewlett–Packard 5890A gas chromatograph fitted with a Supelco SP 2330 fused-silica capillary column (30 m × 0.2 mm i.d.) connected via a capillary direct interface with a Hewlett–Packard 5970 series mass-selective detector, ionizing potential 70 eV. The g.l.c. temperature was 180°, isothermal.

Thermal degradation of sucrose and isolation of products. — Powdered sucrose (Baker analyzed reagent; 6.00 g) was dissolved in deionized water (4.0 mL) together with citric acid (60 mg). The water was then mostly removed (80–85%) at room temperature under 0.1 mmHg, leaving a solid sponge of amorphous sucrose. The sample was heated in an oil-bath (100 ±0.1°) under the same vacuum for 60 min. Within 10–15 min, the

solid sponge had collapsed to a viscous liquid which, on eventual cooling, hardened to a transparent pale yellow-brown glass. This was dissolved in water (30 mL) and abs. EtOH (30 mL) was added. Acetone (220 mL) was then added dropwise with stirring until a white precipitate formed, which on standing settled as an oily deposit from which the clear supernatant was decanted. The precipitate was dried under vacuum and then dissolved in water for preparative l.c.

A similar sample of the precipitate (4 g) was dissolved in water (15.0 mL), solid yeast invertase (Sigma, 825 units/mg; 6.3 mg) added, and the sample incubated at 37°. After 30 min the oligosaccharides were precipitated as previously using EtOH (15.0 mL) and acetone (240 mL). L.c. (system *b*) of this product revealed that all of the sucrose and most of the product oligomers had been degraded to glucose and fructose (Fig. 3). There remained two components, apparently resistant to invertase, and a new substance had formed (* in Fig. 3).

Identification of products **10, 12, 13,** *and* **14** *of thermal degradation.* — From the precipitate that had not been treated with invertase, four components were isolated as amorphous solids by l.c. Two of these gave single peaks when co-injected with authentic samples of 6-kestose (**10**) and neokestose (**13**), respectively, in both l.c. systems. Methylation analysis was consistent with these assignments (Table IV). Of the remaining two products, one was not coincident in l.c. with any known kestose. Methylation analysis (Table IV) showed it to be isomeric with 6-kestose, and ^{13}C-n.m.r. (Table V) indicated the presence of an α-linked fructofuranoside. This product was one of the invertase-resistant components, and on the basis of this evidence it was named *iso-6-kestose* (**12**, f.a.b.–m.s. M + 1 = 505.1816. Calc. for $C_{18}H_{33}O_{16}$ 505.1769), $[\alpha]_D^{22}$ + 40.5° (*c* 1, D_2O).

Methylation analysis and ^{13}C n.m.r. of the final component indicated that it was a mixture of 1-kestose and an isomer of neokestose, which were coincident on both l.c. systems. F.a.b.–m.s. of the mixture (M + 1 = 505.1803) was consistent with this conclusion. Treatment of this mixture with invertase and examination by l.c. showed the formation of glucose and fructose, together with the same new substance observed from the total product-mixture after enzyme treatment. This new substance (5 mg) was isolated by l.c. as an amorphous solid and reduced in aqueous solution (0.25 mL) with $NaBD_4$ (5 mg in 0.25 mL). The mixture was acidified by shaking with Amberlite resin IRC 50 (H^+) (1 mL), filtered, and the filtrate blown dry repeatedly with MeOH (6 × 1 mL) at 25°. The reduced product was then subjected to methylation analysis, which gave 1,3,4,5-tetra-*O*-methyl-D-fructose diacetate and 6-*O*-acetyl-1,2,3,4,5-penta-*O*-methyl-D-(1-^{2}H)glucitol. The mass spectrum of the latter compound is not included in Table III because of its structural dissimilarity from the other reference compounds. The major fragment-ions were: 101 (100%), 117 (53), 90 (30), 75 (26), 102 (24), 60 (19), 161 (16), 146 (16), 88 (16), and 87 (13). ^{1}H-N.m.r. spectroscopy of the original substance (D_2O reference 1,4-dioxane, 3.74 p.p.m., 350 MHz) revealed a doublet (*J* 3.7 Hz) at 5.20 p.p.m. (C-1α) and another doublet (*J* 7.9 Hz) at 4.63 p.p.m. (C-1β), in the integrated ratio of 1:1.56. The ^{13}C-n.m.r. spectrum (D_2O, Table V) was also consistent with an equilibrium mixture of glucose anomers to which the fructofuranosyl moiety is α-linked. The methylation analysis shows that fructose is linked to the 6-position of glucose, and

the product was *6-O-(α-D-fructofuranosyl)-D-glucose*, $[\alpha]_D^{22}$ +57.6° (*c* 1, D_2O), f.a.b.–m.s. M + 1 = 343.1227, calc. for $C_{12}H_{22}O_{11}$ 343.1240. As the 1-kestose in the original two-component mixture would be degraded completely by invertase (Table VII), this reducing disaccharide must result from the other trisaccharide which is l.c.-coincident with 1-kestose. This other component was therefore identified as iso-neokestose (**14**).

Invertase treatment of the oligosaccharide thermolysis products was stated above to yield three products after l.c. separation. Of these, one proved to be iso-6-kestose (**12**) and one, the 6-*O*-(α-D-fructofuranosyl)-D-glucose arising from iso-neokestose (**14**). The third component, isolated as an amorphous solid, had $[\alpha]_D^{22}$ +62.2° (*c* 1, D_2O), and on methylation analysis proved to be isomeric with 1-kestose; ^{13}C n.m.r. (Table V) indicated an α-linked fructofuranoside moiety. This product was therefore designated as *iso-1-kestose* (**10**); f.a.b.–m.s. M + 1 = 505.1734, calc. for $C_{18}H_{33}O_{16}$ 505.1769.

Relative rates of formation of kestoses. — Small samples (~70 mg) of amorphous sucrose containing 1% citric acid were heated under vacuum in an oil-bath (100 ±0.1°) for various times. Kestose products were determined from integrated l.c. peak areas by comparison with an external standard of sucrose using the following relative response factors previously determined with pure compounds: 6-kestose, 0.64; neokestose, 0.71; and iso-1-kestose, 0.92.

Reaction of kestoses with β-fructofuranosidase. — Kestose solutions (200 μL; ~10 mg/mL) were incubated with yeast invertase (Sigma; 50 μL; 2700 units/mL) at 37°. The rate of disappearance of kestose was monitored by l.c. and is reported in Table VII.

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REFERENCES

1 G. N. Richards, *Int. Sugar. J.*, 88 (1986) 145–148.
2 G. N. Richards and F. Shafizadeh, *Aust. J. Chem.*, 31 (1978) 1825–1832.
3 W. Moody and G. N. Richards, *Carbohydr. Res.*, 97 (1981) 247–255.
4 D. Bollmann and S. Schmidt-Berg, *Z. Zuckerind. Boehm.*, 15 (1965) 179–265.
5 O. Kandler and H. Hopf, in F. A. Loewus and W. Tanner (Eds.), *Encyclopedia of Plant Physiology*, New Series Vol. 13A, Springer-Verlag, Berlin, 1982, pp. 348–383.
6 H. G. Pontis and E. del Campillo, in P. M. Dey and R. A. Dixon (Eds.), *Biochemistry of Storage Carbohydrates in Green Plants*, Academic Press, New York, 1985, pp. 205–227.
7 J. Edelman and T. G. Jefford, *New Phytol.*, 67 (1968) 517–531.
8 T. Oku, T. Tokunaga, and N. Hosoya, *J. Nutr.*, 114 (1984) 1574–1581.
9 S. C. Ziesenitz and G. Siebert, *J. Nutr.*, 117 (1987) 846–851.
10 H. Hidaka and M. Hirayama, U. K. Pat. application 8616262 (July 3, 1986); H. Hidaka, M. Hirayama, and N. Sumi, *Agric. Biol. Chem.*, 52 (1988) 1181–87.
11 N. C. Carpita, J. Kanabus, and T. L. Housley, *J. Plant Physiol.*, 134 (1989) 162–168.
12 G. G. S. Dutton, *Adv. Carbohydr. Chem. Biochem.*, 30 (1976) 9–110.
13 B. Lindberg, J. Lönngren, and J. L. Thompson, *Acta Chem. Scand.*, 27 (5) (1973) 1819–1821.
14 D. C. DeJongh and K. Biemann, *J. Am. Chem. Soc.*, 85 (1963) 2289–2294.
15 N. K. Kochetkov and O. S. Chizhov, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
16 S. Karady and S. H. Pines, *Tetrahedron*, 26 (1970) 4527–4536.

17 N. K. Kochetkov and O. S. Chizov, *Tetrahedron*, 21 (1965) 2029–2047.
18 J. Lönngren and S. Svensson, *Adv. Carbohydr. Chem. Biochem.*, 29 (1974) 41–106.
19 C.-K. Lee, *Org. Mass Spectrom.*, 22 (1987) 553–556.
20 W. Moody and G. N. Richards, *Carbohydr. Res.*, 108 (1982) 13–22.
21 F. G. Carpenter and E. J. Roberts, *Proc. Tech. Sess. Cane Sugar Refin. Res.*, (1974) 106–115.
22 R. Khan, *Adv. Carbohydr. Chem. Biochem.*, 33 (1976) 236–294.
23 P. C. Ivin and M. L. Clarke, *J. Chromatogr.*, 408 (1987) 393–398.
24 I. Ciucanu and F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
25 J. G. Buchanan, D. A. Cummerson, and D. M. Turner, *Carbohydr. Res.*, 21 (1972) 283–292.
26 H. C. Jarrell, T. F. Conway, P. Moyna, and I. C. P. Smith, *Carbohydr. Res.*, 76 (1979) 45–57.
27 K. Bock and H. Thøgersen, *Ann. Rep. N.M.R. Spectrosc.*, 13 (1982) 1–57.
28 K. Bock, C. Pedersen, and H. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 193–225.
29 T. M. Calub, A. L. Waterhouse, and N. J. Chatterton, *Carbohydr. Res.*, 199 (1990) 11–17.