Note

The identification by ^{1}H- and ^{13}C-n.m.r. spectroscopy of sucrose, 1-kestose, and neokestose in mixtures present in plant extracts

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In the food industry, a knowledge of the presence and proportions of certain sugars in vegetables is important. In seeking to ascertain the presence of sucrose, 1-kestose (**1**, isokestose), and neokestose (**2**) in enriched mixtures extracted from vegetables without isolating the individual sugars, we have found that ^{13}C- and, to a limited extent, ^{1}H-n.m.r. spectroscopy is the method of choice. The analysis depends on the unequivocal assignment of the n.m.r. spectra.

The relevant ^{1}H- and the complete ^{13}C-n.m.r. data for sucrose, 1-kestose, and neokestose are given in Tables I and II, respectively.

^{1}H-N.m.r. data for sucrose have been published[1,2], but because of the complexity of the spectra, only partial data for isokestose and neokestose have been obtained. However, these data are useful.

^{1}H-N.m.r. data for sucrose octa-acetate and 1-kestose hendeca-acetate have been reported[3], as have those for trimethylsilylated sucrose, 1-kestose, 6-kestose, and neokestose[4]. Because of the complexity of the spectra, only the relevant data extracted from the spectrum of 1-kestose and neokestose are given. The data for neokestose were extracted from the spectrum of an enriched extract of onion that contained 1-kestose (**1**) and neokestose (**2**) in the ratio 3:1. The presence of neokestose and 1-kestose in onions has been shown by several authors[5–7].

The chemical shift of the H-1 resonance of the α-D-glucopyranosyl moieties are δ 5.44 (1-kestose), 5.42 (sucrose), and 5.40 (neokestose). The resonance of H-3 in neokestose is at 0.05–0.06 p.p.m. to lower field than the corresponding resonance for the two other oligosaccharides.

The chemical shifts of the H-3,4 resonances of the β-D-fructofuranosyl moieties are diagnostic, namely, 4.25 (d) and 4.06 (t) for sucrose, δ 4.28 and 4.22 (2 d for H-3) and

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δ 4.08 and 4.05 (2 t for H-4) for 1-kestose, and δ 4.19 (d, 2 H for H-3) and δ 4.15 and 4.08 (2 t for H-4) for neokestose. Identification on the basis of these limited ^{1}H-n.m.r. data is realistic.

The ^{13}C-n.m.r. data for sucrose, 1-kestose, and nystose have been published[8-10]. The partial data for neokestose[5], namely, for C-1 of the α-D-glucopyranosyl moiety and C-2 of both the β-D-fructofuranosyl moieties, cannot be used for structural verification. The survey[11] of ^{13}C-n.m.r. data of sugars that occur in food does not contain data for neokestose.

The presence of reducing D-glucose or D-fructose is indicated by the characteristic resonances at δ 96.7 and 92.9 for C-1 in α- and β-D-glucopyranose, respectively, and at δ 102.3 and 98.9 for C-2 of β-D-fructopyranose and β-D-fructofuranose, respectively[12-14].

TABLE I

^{1}H-N.m.r. chemical shifts (δ) (D$_2$O, internal Me$_4$Si) of sucrose, 1-kestose (**1**), and neokestose (**2**)

Compound	α-D-Glucopyranosyl			β-D-Fructofuranosyl	
	H-1	H-2	H-3	H-3(3')	H-4(4')
Sucrose	5.42	3.57	3.48	4.25	4.06
1-Kestose	5.44	3.57	3.47	4.28,4.22	4.08,4.05
Neokestose	5.40	3.57	3.53	4.19,4.19	4.15,4.08

TABLE II

^{13}C-N.m.r. chemical shifts (D$_2$O, internal 1,4-dioxane, 67.4 p.p.m.) of sucrose, 1-kestose (**1**), and neokestose (**2**)

Group	Atom	Sucrose[a]	1-Kestose	Neokestose
α-D-Glucopyranosyl	C-1	92.9	93.3	92.8
	C-2	71.9	71.9	71.8
	C-3	73.4	73.4	73.2
	C-4	70.0	70.0	70.0
	C-5	73.2	73.2	72.4
	C-6	61.0	60.9	60.9[b]
β-D-Fructofuranosyl	C-1	62.5	61.7	61.0[b]
	C-2	104.5	104.1	104.5
	C-3	77.8	77.5	77.1
	C-4	75.4	75.2	75.2
	C-5	82.2	81.9	81.9
	C-6	63.2	62.9	63.2
β-D-Fructofuranosyl	C-1'		61.2	61.1[b]
	C-2'		104.5	104.5
	C-3'		77.5	77.6
	C-4'		74.7	74.7
	C-5'		82.0	82.1
	C-6'		63.1	63.2

[a] Taken from ref. 1. [b] May be interchanged.

The absence of these resonances indicates that only non-reducing sugars are present.

The ^{13}C-n.m.r. chemical shifts reported for sucrose were verified by 2-D INADE-QUATE[15] and can be assigned to the kestoses unequivocally. The spectra of the trisaccharides contain some easily recognisable resonances, e.g., those of C-3,4,5 of the β-D-fructofuranosyl moieties are well differentiated pairs of resonances. The C-2 resonances of the β-D-fructofuranosyl moieties of 1-kestose have different chemical shifts (δ 104.5 and 104.1), whereas those of neokoestose are identical (δ 104.5).

The region δ 61–63 is a fingerprint region. For sucrose, the resonance of C-6 of the α-D-glucopyranosyl group is at δ 61.0 and those of C-1 and C-6 of the β-D-fructofuranosyl group are at δ 62.5 and 63.2, respectively; the chemical shift of the resonance of C-1 is characteristic for sucrose. For 1-kestose, the resonances at δ 60.9, 61.2, and δ 61.7 are assigned to C-6 of the α-D-glucopyranosyl group and C-1 of the two β-D-fructofuranosyl moieties, respectively. For neokestose, the corresponding resonances were at δ 60.9, 61.0, and 61.1.

In the region δ 62.9–63.5, the resonances of C-6 of β-D-fructofuranosyl moieties in 1-kestose were at δ 62.9 and 63.1, and for neokestose only at δ 63.2.

For (1→n)-linked aldohexo- and aldopento-pyranoses[8], in comparison with the parent sugars, the resonances of the carbons involved in the linkage are shifted downfield by 5–9 p.p.m., whereas those of the neighbouring carbons are shifted upfield by 1–2 p.p.m. Where an aldohexopyranose is 2-linked to β-D-fructofuranose, only small shifts of these resonances occur. Thus, the rule applied to identify the position of the linkage does not hold when C-2 of β-D-fructofuranose is involved. The data relevant to the present study are given in Table III. Because of the different sources of the data, only shifts >1 p.p.m. are considered.

For sucrose, the resonance of C-1 of the α-D-glucopyranosyl group is not shifted, whereas those of C-1 and C-2 of the β-D-fructofuranosyl group are shifted upfield (−1.1 p.p.m.) and downfield (+1.9 p.p.m.), respectively. For 1-kestose, there is a small downfield shift for the resonance of C-2 (+1.5–+1.9 p.p.m.) and a small upfield shift for that of C-1 (−1.9––2.4 p.p.m.). These data indicate that a (1→2) linkage between two β-D-fructofuranosyl moieties causes shifts comparable to those for a (1→2) linkage between α-D-glucopyranosyl and β-D-fructofuranosyl moieties. When (1→2) and (2→1) linkages occur on the same β-D-fructofuranosyl moiety, a smaller downfield shift is seen for the resonance of C-2. For neokestose, the chemical shift of the resonance of C-1 of the α-D-glucopyranosyl moiety shows almost no shift. The resonances of C-2 of both of the β-D-fructofuranosyl groups show the same downfield shift (+1.9 p.p.m.) and those of C-1 are shifted upfield (−2.5––2.6 p.p.m.). The resonance of C-6 of the α-D-glucopyranosyl moiety, for which a downfield shift of 5–8 p.p.m. would be expected if the linkage involves two pyranose units, shows a small upfield shift. In this respect, in α-blastose [α-D-fructofuranosyl-(2→6)-α-D-glucopyranose], only a minor shift was found for the resonance of C-6 of the α-D-glucopyranosyl moiety[8].

The data in Table II show that, for the α-D-glucopyranosyl moieties in sucrose and neokestose, the resonance of C-1 is at δ 92.8–92.9, whereas, for 1-kestose, it is at δ 93.3. Likewise, the resonance of C-5 is at δ 73.2–73.4 for the former compounds and at δ 72.4 for the latter.

TABLE III

Chemical shifts ($\Delta\delta$) for ^{13}C resonances

Compound	α-D-Glucopyranosyl		β-D-Fructofuranosyl	
	C-1	C-6	C-1	C-2
α-D-Glucopyranose[a]	92.9	61.6		
β-D-Fructofuranose[b]			63.6	102.6
Sucrose[b]	92.9	61.0	62.5	104.5
	(−)	(−0.6)	(−1.1)	(+1.9)
1-Kestose[c]	93.3	60.9	61.7	104.1
	(+0.4)	(−0.7)	(−1.9)	(+1.5)
			61.2	104.5
			(−2.4)	(+1.9)
Neokestose[c]	92.8	60.9	61.0	104.5
	(−0.1)	(−0.7)	(−2.6)	(+1.9)
			61.1	104.5
			(−2.5)	(+1.9)
α-Blastose[b]	93.0	61.7	61.1	104.6
	(+0.1)	(+0.1)	(−2.5)	(+2.0)

[a] Taken from ref. 12. [b] Taken from ref. 8. [c] Taken from Table II.

The data for the β-D-fructofuranosyl moieties are informative. In addition to the data for the C-2 and C-6 resonances mentioned above for 1-kestose, the two C-3 resonances of the β-D-fructofuranosyl moieties are at δ 77.5. For sucrose, this resonance is found at δ 77.8 and, for neokestose, at δ 77.1 and 77.6. In the trisaccharides, the C-4 resonances of this moiety are found at δ 75.2 and 74.7, and, in sucrose, at δ 75.4.

Except for the resonance of C-6 of the β-D-fructofuranosyl group linked to the α-D-glucopyranosyl moiety in neokestose, the corresponding resonances of both β-D-fructofuranosyl moieties in 1-kestose and neokestose were not differentiated here or elsewhere[9].

Thus, the region δ 61–63.2 can be used to differentiate between sucrose, 1-kestose, and neokestose in a mixture, as summarised in Scheme 1.

EXPERIMENTAL

The n.m.r. spectra were obtained with a Bruker AM500 spectrometer, operating in the F.t. mode with quadrature detection. For the ^1H-n.m.r. spectra (500.137 MHz), a pulse width of 8 μs was used with an acquisition time of 3.277 s and a resolution of 0.305 Hz/point. For the ^{13}C-n.m.r. spectra (125.7 MHz), the resonance of 1,4-dioxane (δ 67.4) was used as an absolute value, a pulse width of 8 μs was used, and a resolution of 1.907 Hz/point.
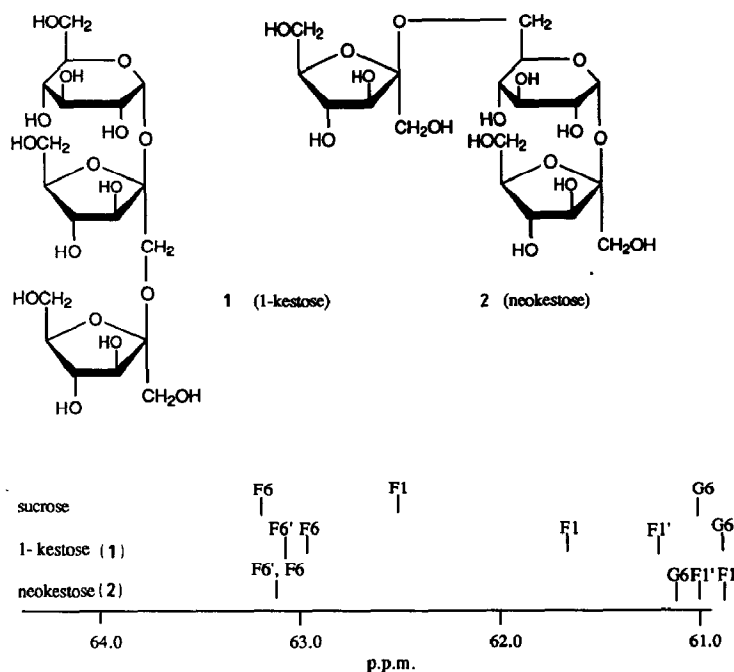
Scheme 1. Diagrammatic representation of the diagnostic ^{13}C resonances (see Table II for chemical shift data); F6 connotes C-6 in the fructose moiety *etc.*

The ^{13}C-n.m.r. data in Table II were obtained for solutions in D_2O (internal 1,4-dioxane, 67.4 p.p.m.). In Table II, the known data of sucrose and 1-kestose[8,9] have been used *vs.* our reference of choice, so that the values listed here are 0.4–0.5 p.p.m. to higher field. The data for 1-kestose were obtained on a pure sample obtained from an enzymic reaction on sucrose. The data for neokestose were obtained from a mixture of neokestose and 1-kestose as extracted from onions.

Extraction of fructose oligosaccharides from vegetables. — Vegetables (onion, leek, garlic, asparagus, *etc.*) were sliced into small pieces with an average cross-section of < 3 mm, by means of a knife in order to minimise damage of the cell walls and consequent hydrolysis by carbohydrases. The slices were immersed immediately in 4:1 EtOH–H_2O. The volume of EtOH was at least five times that of the vegetables, and the mixture was boiled under reflux for 1 h. The amount of fructose oligosaccharides in the extract was determined by h.p.l.c. on two columns (30 cm × 47 mm) of Aminex HPX87K (K^+ form) (particle size, 9 μm) in series, by elution at 0.6 mL/min with H_2O at pH 9.5 (KOH) and 85°. Since ethanol interferes with the sugar analysis, the alcohol was removed in a stream of air (15 h) after addition of NaN_3 (100 p.p.m.).

Fractions enriched in oligosaccharides of d.p. 3 were obtained by using a Varian 5500 LC pump and a UV 200 variable wavelength detector, a Valco CI6W injector (equipped with a 200-μL loop), a column (250 × 10 mm) filled with 10-μm RSiL C_{18}

(Bio-Rad), and elution at room temperature with H_2O for 20 min, then by a linear gradient to 100% of MeCN for 3 min at 3 mL/min. The H_2O and MeCN were h.p.l.c. grade. Detection was at 195 nm.

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