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PHYSICAL STUDIES ON OLIGOSACCHARIDES RELATED TO SUCROSE.

PART I. N.M.R. STUDIES ON THE PERACETATES OF SUCROSE, 1-KESTOSE, AND NYSTOSE*

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ABSTRACT

The n.m.r. spectra of sucrose octaacetate (**1**), 1-kestose hendecaacetate (**2**), and nystose tetradecaacetate (**3**) were measured in benzene-d_6, chloroform-*d*, and acetone-d_6, at 100 and 220 MHz. Signals of H-1, 2, 3, and 4 of the α-D-glucopyranosyl residue, and of H-3 and H-4 of the β-D-fructofuranosyl residue(s), could be observed individually for each of the compounds. The data confirm that all D-fructose residues in 1-kestose and nystose are in the furanoid ring-form. Observation of a two-proton, AB system near τ 6.3 (chloroform-*d*) in the spectrum of **2** provides diagnostic evidence for the (2→1) linkage between the two D-fructofuranosyl residues in 1-kestose [β-D-Fru*f*-(2→1)-β-D-Fru*f*-(2↔1)-α-D-G*p*]; other conceivable linkages are excluded. A second such AB system, observed in the spectrum of **3**, establishes that nystose is built up from 1-kestose by (2→1) attachment of an additional D-fructofuranosyl group. Other points of attachment are excluded, and the structure β-D-Fru*f*-(2→1)-β-D-Fru*f*-(2→1)-β-D-Fru*f*-(2↔1)-α-D-G*p*, previously suggested for nystose, is confirmed. The n.m.r. signals for H-2, 3, and 4 of the α-D-glucopyranosyl residue in **1**, **2**, and **3**, and also in methyl α-D-glucopyranoside tetraacetate (**4**), showed close correlation.

INTRODUCTION

Sucrose can be regarded as the biogenetic precursor of a family of oligosaccharides found in sugar-cane and sugar-beet products. A molecule of sucrose acts as the acceptor, and sucrose provides the source of D-fructofuranosyl groups, for the enzyme-catalyzed generation of homologous D-fructofuranosyl derivatives of sucrose, as shown in Scheme I. Transfer of a β-D-fructofuranosyl group to O-6 of the D-fructosyl moiety of sucrose gives the trisaccharide 6-kestose[1,2], and transfer to O-6 of the

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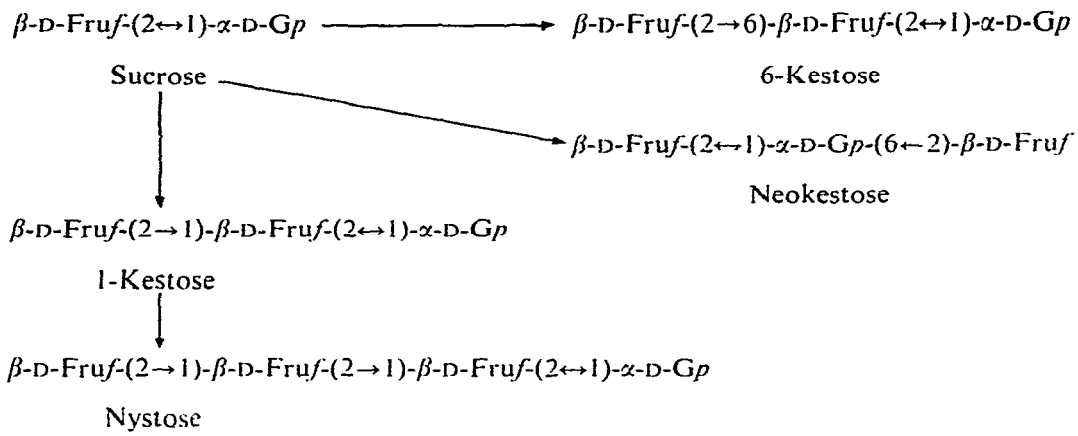


Scheme I

D-glucosyl moiety gives neokestose[2]. Transfer of the β-D-fructofuranosyl group to O-1 of the D-fructosyl moiety of sucrose gives the trisaccharide 1-kestose[2,3], and repetition of this process on the new terminal D-fructofuranosyl group gives the tetrasaccharide nystose[4]. The polysaccharide inulin can be regarded as resulting from an extension of the process exemplified by sucrose→1-kestose→nystose.

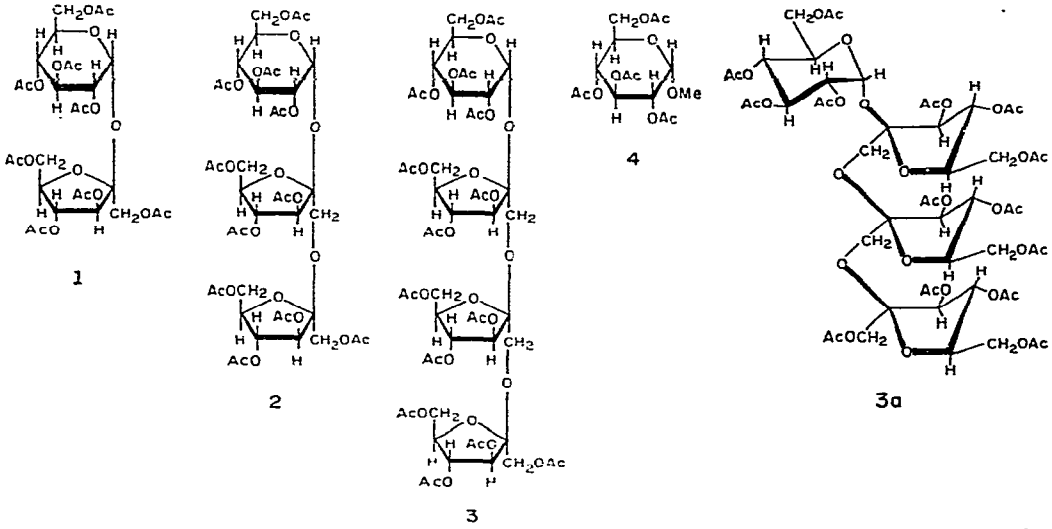
The linkage sequence in 1-kestose (see Scheme I) has been established by methylation and by partial hydrolysis[3], and the configurations of the linkages have been assigned on the basis of enzymic[3] and optical rotatory[4] evidence. Nystose, one of the products of the action of a fungal *alpha*-amylase on sucrose, has been shown[4] by fragmentation analysis and periodate oxidation to be an *O*-D-fructofuranosyl derivative of 1-kestose; the β-D linkage is indicated by optical rotatory data. By consideration of the normal specificity of the transglycosylase that produces nystose, the new β-D-fructofuranosyl group was presumed to be linked through O-1 of the terminal D-fructofuranosyl group of 1-kestose, to give the structure shown in Scheme I for nystose, but the position of this linkage has not been verified by a direct procedure.

As part of a broader program on the characterization, by modern physical methods, of oligosaccharides related to sucrose, the present article records a detailed study by n.m.r. spectroscopy at 100 and 220 MHz of the peracetates of sucrose, 1-kestose, and nystose. The data provide direct confirmation of the linkage positions in the structure already proposed for nystose.

DISCUSSION

The n.m.r. spectra of acetylated sugars can, in general, be subdivided[5] into three nonoverlapping regions, one of which is the acetyl-proton region near τ 8.0. The remaining protons, attached to the carbon atoms of the sugar residues, give rise to two discrete groups of signals. Signals appearing at fields higher than τ 5.5 arise from methylene groups and from protons of C_2CHOR (R ≠ Ac) groups (such as H-5 of hexopyranoses and hexulofuranoses), whereas resonances below τ 5.4 may

be assigned to the remaining methine protons (of C_2CHOAc groups, and anomeric protons, O_2CHR). On this basis, broad assignments can be made in regard to the n.m.r. spectra of sucrose octaacetate (**1**), 1-kestose hendecaacetate (**2**), and nystose tetradecaacetate (**3**). In the spectra of **1**, **2**, and **3**, at 100 MHz in chloroform-*d*



(see Fig. 1), the integrated intensities of the lowest-field (methine) groups of signals are 6, 8, and 10 protons, respectively. In each case, this multiplet includes the signals of H-1, H-2, H-3, and H-4 of the D-glucosyl group, together with the signals* of H-3′ and H-4′ of each D-fructose residue present. In the case of methyl α-D-glucopyranoside tetraacetate (**4**), a model compound for the acetylated α-D-glucopyranosyl group in **1**, **2**, and **3**, the H-1, 2, 3, and 4 signals likewise fall in this lowest-field region[6]. The signals for acetyl groups are observed as groups of closely spaced, three-proton singlets (some overlapping), having the anticipated intensities of 24, 33, 42, and 12 protons, for **1**, **2**, **3**, and **4**, respectively.

In the intermediate-field region (above τ 5.5, but below the acetyl-proton region), compound **4** shows signals of three protons (H-5 and two H-6 protons). Sucrose octaacetate (**1**) shows signals of 8 protons, because of 5 additional protons (H-1′$_s$ and H-6′$_s$ methylene protons, and H-5′$_s$) of the D-fructofuranosyl group. Addition of a second D-fructofuranosyl group introduces a further 5 protons resonating in this region, to give a total integral of 13 protons observed with 1-kestose hendecaacetate (**2**). Similarly, the addition of a third D-fructofuranosyl group introduces an additional

*Unprimed numbers refer to protons on the D-glucose moiety, and primed numbers refer to protons on an unspecified D-fructose residue. Protons on the same D-fructose residue are identified by the same letter (H-3a′, H-4a′, for example), except when the individual D-fructose residue is specifically identified. Protons on the D-fructofuranosyl group of sucrose are identified by a subscript "s", protons on the terminal D-fructofuranosyl group of 1-kestose are identified by a subscript "k", and protons on the terminal D-fructofuranosyl group of nystose are identified by the subscript "n".

5 protons, to give a total of 18 protons in the intermediate-field region for nystose tetradecaacetate (3).

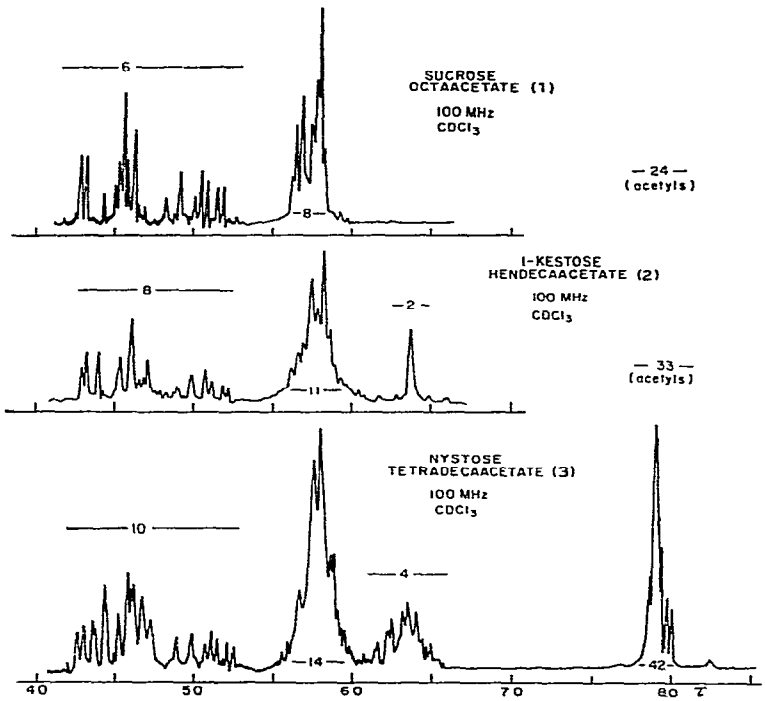


Fig. 1. N.m.r. spectra, at 100 MHz in chloroform-*d*, of sucrose octaacetate (1), 1-kestose hendecaacetate (2), and nystose tetradecaacetate (3); the acetyl-proton region of the spectra of 1 and 2 is omitted. Numbers on the curves denote integrated number of protons.

It is noteworthy that 2 and 3 contain methylene groups of two types, the C–CH_2OAc groups (also present in 1 and 4), together with methylene groups of the type C–CH_2OR (R ≠ Ac) that form bridges between the furanose rings. One such bridge is present in 2, and two are present in 3. The sugar residue (R) is not so strongly deshielding as is an acetyl group; in consequence, the signals of the "methylene bridges" can be observed in the τ 5.9–6.4 region, independent of other signals in the "intermediate" region. In the spectrum of 2 (see Fig. 1), the "intermediate" region shows an 11-proton multiplet together with a 2-proton, apparent singlet for the methylene bridge between the D-fructofuranosyl residues; in the spectrum of 3, the corresponding signals are a 14-proton multiplet together with a 4-proton multiplet for the two methylene bridges.

Detailed analyses of the low-field group of signals have been made by means of multiple-solvent techniques, nuclear-magnetic double-resonance, and the use of spectra measured at very high field-strength (equivalent to 220 MHz for protons). Assignments are presented in Tables I, II, and III, and are discussed in the following sections. The signals of the methylene bridges provide evidence for the linkage-

sequence in **2** and **3**. Other signals in the "intermediate" group are not readily analyzed.

Analysis of the lowest-field group of signals. — A. Sucrose octaacetate (**1**). The 100-MHz spectrum in chloroform-*d* (see Fig. 1) shows a quartet at τ 5.13, having spacings of 3.7 and 9.9 Hz, at the high-field end of this group of signals. These spacings, characteristic[6] of an axial proton coupled to a vicinal, axial proton on one side and a vicinal, equatorial proton on the other, identify the signal as H-2. From the spacings of the H-2 signal, the H-1 signal can be identified as a doublet at lowest field (τ 4.32), and the H-3 and H-4 signals can be identified as wide triplets; these assignments are readily confirmed by irradiating the H-2 signal and observing perturbation of the H-1 and H-3 signals. The remaining signals, a doublet at τ 4.55 and a triplet at τ 4.64, could be assigned to H-$3'_s$ and H-$4'_s$ on the basis of their signal multiplicities. These assignments are in agreement with those of Lemieux and Nagarajan[7] for **1**. It may be noted (see Table IV) that the chemical shifts of H-2, 3, and 4 are closely similar to those[6] of methyl α-D-glucopyranoside tetraacetate (**4**); the shifts of the H-1 signals for **1** and **4** differ, presumably because of the difference in the nature of the aglycon.

Much better separation of signals in this region could be achieved by use of benzene-d_6 as the solvent, and further resolution was obtained by measuring the spectrum at 220 MHz (see Fig. 2). Complete separation of the H-2, H-4, H-$3'_s$, and H-$4'_s$ signals was obtained, and the only overlap was between the H-1 and H-3 signals; as there is no appreciable spin-coupling between these protons, the first-order analysis is applicable. The first-order couplings (see Table II) can be expected to be very close to the absolute values. Relative to the signal positions in chloroform-*d*, the signals appear 0.2–0.4 p.p.m. to lower field in benzene-d_6, but the relative positions of the signals in the field are not changed. The signal positions with acetone-d_6 as the solvent were little different from those observed with chloroform-*d*, but the spectrum was not so well resolved.

B. 1-Kestose hendecaacetate (**2**). The low-field region of the spectrum of **2** in benzene-d_6, measured at 100 and 220 MHz (see Fig. 3), can be analyzed on a first-order basis; similar analyses can be made for the spectra measured in chloroform-*d* and acetone-d_6 (see Table I). The H-2 quartet is readily recognized; and the H-3 and H-4 triplets, and the H-1 doublet, can be identified by their spacings and by spin decoupling. The chemical shifts of H-2, 3, and 4 in chloroform-*d*, and also in benzene-d_6, correspond very closely (see Table IV) for the series methyl α-D-glucopyranoside tetraacetate (**4**), sucrose octaacetate (**1**), and 1-kestose hendecaacetate (**2**).

The remaining signals in the low-field group for **2** can be observed (benzene-d_6 at 220 MHz, Fig. 3) as an isolated doublet at τ 3.95, an isolated triplet at τ 4.38, and a doublet at τ 4.22 very close to the H-3 signal, with a triplet (τ 4.26) overlapping the doublet at τ 4.22. The doublets can be assigned to H-3′ signals, and the triplets to H-4′ signals. The doublet at τ 4.22 and the triplet at τ 4.26 arise from protons on different D-fructofuranose residues, because second-order effects between the two signals are not observed. The assignment of the doublet at τ 4.22 and the triplet at

TABLE I

CHEMICAL SHIFTS OF METHINE AND METHYLENE PROTONS OF PERACETYLATED SUCROSE, 1-KESTOSE, AND NYSTOSE[a]

Compound	*Solvent*	*Chemical shifts, τ*												
		D-*Glucose residue*				D-*Fructose residues*						*Methylene bridges*		*Other protons*
		H-1	*H-2*	*H-3*	*H-4*	*H-3a'*	*H-4a'*	*H-3b'*	*H-4b'*	*H-3c'*	*H-4c'*	*H-1a'*	*H-1b'*	*(integral)*
Sucrose octaacetate (1)	C_6D_6	4.15	4.99	4.22	4.71	4.33[b]	4.48[c]							5.43–5.89 (8)
	$CDCl_3$	4.32	5.13	4.55	4.93	4.55[b]	4.64[c]							5.62–5.95 (8)
	$(CD_3)_2CO$	4.25	5.12	4.48	4.91	4.44[b]	4.55[c]							5.53–5.95 (8)
1-Kestose hendecaacetate (2)	C_6D_6	3.92	4.87	4.15	4.67	4.22	4.38	3.95	4.26			6.01, 6.01		5.30–5.90 (11)
	$CDCl_3$	4.28	5.12	4.58	4.96	4.54	4.68	4.32	4.58			6.29, 6.38		5.58–5.93 (11)
	$(CD_3)_2CO$	4.19	5.10	4.50	4.92	4.47	4.59	4.18	4.49			6.12, 6.28		5.48–5.92 (11)
Nystose tetradecaacetate (3)	C_6D_6	3.87	4.90	4.17	4.65	4.19	4.37	3.98	4.30[d]	3.96	4.27[d]	5.81, 5.99	5.84, 6.00	5.35–5.78 (14)
	$CDCl_3$	4.28	5.17	4.60	4.97	4.55	4.68	4.42	4.67[d]	4.41	4.64[d]	6.22, 6.40	6.24, 6.42	5.54–5.97 (14)
	$(CD_3)_2CO$	4.19	5.11	4.50	4.91	4.48	4.59	4.26	4.54[d]	4.26	4.53[d]	6.05, 6.23	6.08, 6.27	5.51–5.86 (14)

[a]Data taken from 100- and 220-MHz spectra. [b]Identified as H-$3_g'$. [c]Identified as H-$4_g'$. [d]Not specifically differentiated.

TABLE II

FIRST-ORDER COUPLING-CONSTANTS FOR PERACETYLATED SUCROSE, 1-KESTOSE, AND NYSTOSE[a]

Compound	*Solvent*	*Coupling constants[b], Hz*										
		D-*Glucose residue*				D-*Fructose residues*						*Methylene bridges,*
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{3a',4a'}$	$J_{4a',5a'}$	$J_{3b',4b'}$	$J_{4b',5b'}$	$J_{3c',4c'}$	$J_{4c',5c'}$	$J_{geminal}$
Sucrose octaacetate (1)	C_6D_6	3.7	10.5	9.5	9.7	5.5	5.1					
	$CDCl_3$	3.7	9.9	10.0	9.3	5.5	6.0					
	$(CD_3)_2CO$	3.9	10.3	10.6	9.3	5.5	5.0					
1-Kestose hendecaacetate (2)	C_6D_6	3.9	10.3	9.8	10.0	6.5	6.3	8.5	8.1			
	$CDCl_3$	3.9	9.0	9.4	10.0	7.0	6.0	8.0	8.0			9.5
	$(CD_3)_2CO$	3.7	10.5	10.1	9.8	6.0	6.2	7.8	6.9			9.5
Nystose tetradecaacetate (3)	C_6D_6	4.0	10.0	10.0	10.0	6.5	6.2	7.2	~7	7.2	~7	9.8, 9.6
	$CDCl_3$	3.5	10.0	9.5	9.8	6.8	~7	7.8	~7	7.8	~7	9.8, 9.5
	$(CD_3)_2CO$	4.0	10.6	9.5	10.0	6.4	~6.5	7.5	~7	7.5	~7	9.9, 9.4

[a]Data taken from 100- and 220-MHz spectra. [b]Values are considered accurate to ±0.5 Hz or better.

TABLE III

CHEMICAL SHIFTS OF ACETATE RESONANCES OF PERACETYLATED SUCROSE, 1-KESTOSE, AND NYSTOSE[a]

Compound	*Solvent*	*Chemical shifts of peaks observed, τ (integral)*
Sucrose octaacetate (1)	C_6D_6	8.07(3), 8.10(6), 8.17(3), 8.20(3), 8.25(3), 8.27(3), 8.32(3)
	$CDCl_3$	7.82(3), 7.88, 7.90(15), 7.95(3), 7.98(3)
	$(CD_3)_2CO$	7.86(3), 7.95(12), 7.98(3), 8.01(3), 8.04(3)
1-Kestose hendecaacetate (2)	C_6D_6	8.01(3), 8.02(3), 8.07(3), 8.10(3), 8.11(3), 8.13(3), 8.16(3), 8.25(3), 8.26(3), 8.33(3), 8.36(3)[b]
	$CDCl_3$	7.88(3), 7.91, 7.92(6), 7.93(12), 7.98(6), 8.01(3), 8.03(3)
	$(CD_3)_2CO$	7.87(3), 7.90(3), 7.93, 7.94(9), 7.96, 7.97(12), 8.02(3), 8.05(3)
Nystose tetradecaacetate (3)	C_6D_6	7.92(3), 7.99(3), 8.00(3), 8.01(3), 8.04(3), 8.12(9), 8.16(3), 8.27(3), 8.28(3), 8.32(3), 8.38(3), 8.39(3)[b]
	$CDCl_3$	7.87(6), 7.91(12), 7.92(12), 7.93(6), 7.95(6), 7.98(3), 8.02(3)
	$(CD_3)_2CO$	7.88(3), 7.90(9), 7.92(3), 7.95(3), 7.97(12), 8.00(3), 8.01(3), 8.03(3), 8.05(3)

[a]From 100-MHz spectra, unless otherwise stated. [b]Clear resolution of signals separated by 0.01 p.p.m. observed in 220-MHz spectra.

TABLE IV

COMPARATIVE, CHEMICAL-SHIFT DATA FOR THE D-GLUCOPYRANOSYL GROUP IN THE PERACETATES OF METHYL α-D-GLUCOPYRANOSIDE, SUCROSE, 1-KESTOSE, AND NYSTOSE

Compound No.	*Chemical shifts, τ*							
	Chloroform-d				*Benzene*-d_6			
	H-1	*H-2*	*H-3*	*H-4*	*H-1*	*H-2*	*H-3*	*H-4*
4[a]	(5.04)	5.11	4.51	4.93	(5.11)	4.96	4.20	4.73
1	4.32	5.13	4.55	4.93	4.15	4.99	4.22	4.71
2	4.28	5.12	4.58	4.96	3.92	4.87	4.15	4.67
3	4.28	5.17	4.60	4.97	3.87	4.90	4.17	4.65

[a]Data from ref. 6.

τ 4.38 to H-3a′ and H-4a′, respectively, of one D-fructofuranosyl residue, and the signals at τ 3.95 and 4.26 to H-3b′ and H-4b′ of the D-fructofuranosyl residue, is also supported by spin-coupling data (see Table II); the first pair (*a*) show $J_{3a',4a'} = 6.5$ Hz,

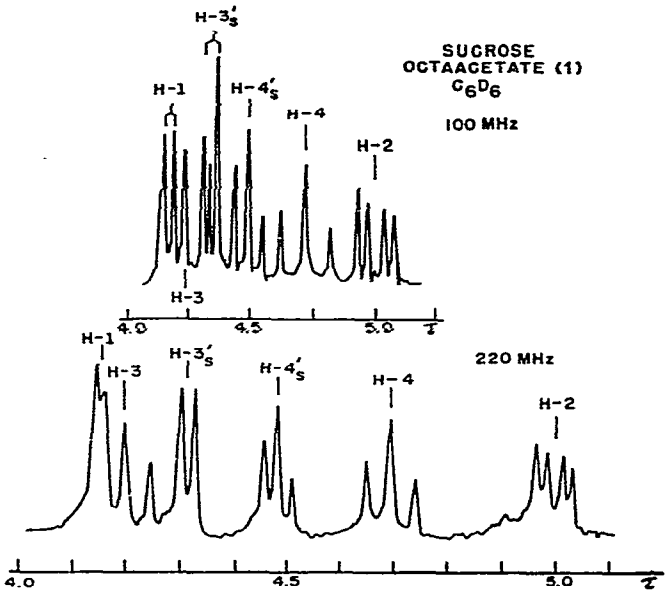


Fig. 2. The low-field portion of the n.m.r. spectrum of sucrose octaacetate (**1**) in benzene-d_6, at 100 and 220 MHz.

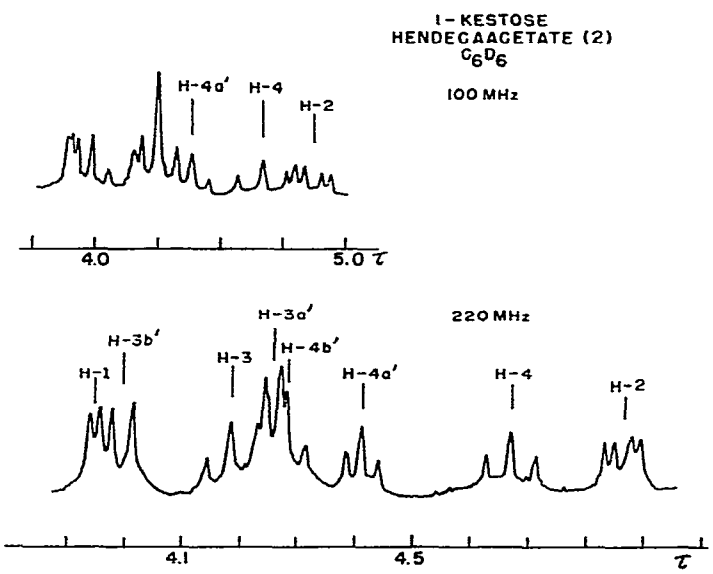


Fig. 3. The low-field portion of the n.m.r. spectrum of 1-kestose hendecaacetate (**2**) in benzene-d_6 at 100 and 220 MHz.

and the second pair (*b*) show $J_{3b',4b'} = 8.5$ Hz. The $J_{3s',4s'}$ coupling in sucrose octaacetate (**1**) in benzene-d_6 is 5.5 Hz. Similar groupings, in pairs, for H-3a′ and H-4a′, and H-3b′ and H-4b′, can be made from spectra measured in chloroform-*d* and acetone-d_6 (see Tables I and II), but the spectra are not so well resolved, and are readily analyzed only at 220 MHz.

It might be suggested that, by correlation with the chemical shifts and couplings observed with sucrose octaacetate (**1**) for H-3′$_s$ and H-4′$_s$, the H-3a′ and H-4a′ pair in **2** may be identified as H-3′$_s$ and H-4′$_s$, and the H-3b′ and H-4b′ pair as H-3′$_k$ and H-4′$_k$. However, it could equally well be argued that the shifts and couplings observed in **1** for H-3′$_s$ and H-4′$_s$, and in **2** for H-3a′ and H-4a′, are characteristic of a terminal D-fructofuranosyl group. Definitive assignment would require study of the spectrum of a sample of **2** that had been specifically deuterated[6,8] in one of the D-fructofuranosyl residues.

C. Nystose tetradecaacetate (**3**). The 100-MHz spectra were difficult to analyze completely in the low-field region, but the 230-MHz spectrum in benzene-d_6 (see Fig. 4), and the 220-MHz spectra in acetone-d_6 (see Fig. 5) or chloroform-*d*, showed the H-1, H-2, and H-4 signals separated from other signals, and the H-3 triplet could be extracted from a group of overlapping signals. The chemical shifts of H-1, 2, 3, and 4 in chloroform-*d*, benzene-d_6, and acetone-d_6 were almost identical with those of the corresponding signals observed for the lower homologs **2** and **1** (see Tables I and IV); the values for H-2, 3, and 4 were also very close to those observed with the monomer model **4** in the two solvents (chloroform-*d* and benzene-d_6) for which comparative data are available.

The H-3′ and H-4′ signals for the three D-fructofuranosyl residues in **3** were identified from the spectrum in benzene-d_6 (see Fig. 4) as doublets at τ 3.96, 3.98, and 4.19, and as triplets at τ 4.27, 4.30, and 4.37. From coupling data and the absence of second-order effects, the doublet at τ 4.19 and the triplet at τ 4.37 could be assigned to the same D-fructofuranosyl residue (H-3a′ and H-4a′). The doublets at τ 3.96 and 3.98 had almost identical splittings, so that correlation of each with the individual triplets at τ 4.27 and 4.30 (to differentiate the pairs H-3b′ and H-4b′, and H-3c′ and H-4c′) could not be made.

The pair of signals H-3a′ and H-4a′ have chemical shifts considerably different from those for the other two pairs (H-3b′ and H-4b′, H-3c′ and H-4c′), suggesting that H-3a′ and H-4a′ arise from the terminal D-fructofuranosyl group (H-3′$_n$ and H-4′$_n$), and the other two pairs arise from the inner D-fructofuranosyl residues (H-3′$_k$ and H-4′$_k$, H-3′$_s$ and H-4′$_s$), but this rationalization cannot be proved on the basis of the data at present available.

The H-3b′ and H-3c′ signals were observed, in chloroform-*d* at 220 MHz, as low-field doublets of almost identical chemical-shifts; in acetone-d_6 (see Fig. 5), the two signals coincided exactly. The signals of H-3a′, H-4a′, H-4b′, and H-4c′ were more difficult to identify in the spectra measured in chloroform-*d* or acetone-d_6 than in the spectra in benzene-d_6, because of more extensive overlap of signals in the first two solvents.

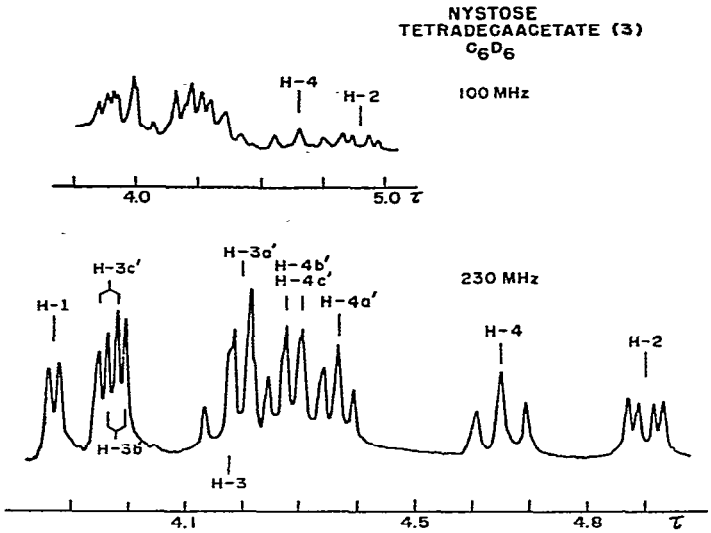


Fig. 4. The low-field portion of the n.m.r. spectrum of nystose tetradecaacetate (3) in benzene-d_6 at 100 and 230 MHz.

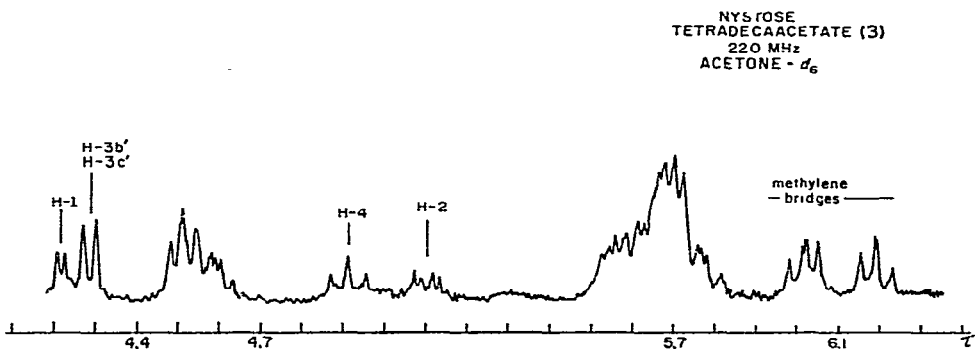


Fig. 5. The low-field portion of the 220 MHz spectrum of nystose tetradecaacetate (3) in acetone-d_6.

Coupling constants. — The coupling constants $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ observed for the D-glucopyranosyl group in **1**, **2**, and **3** (see Table II) are unexceptional; they closely resemble the couplings observed[6] for methyl α-D-glucopyranoside tetraacetate (**4**), and accord entirely with the anticipated *Cl*(D) conformation of the pyranoid ring in **1**, **2**, **3**, and **4**. Some variation in the values of $J_{3',4'}$ for the D-fructofuranose residues is noted with **1**, **2**, and **3**, probably representing minor deviations from a favored conformation[7] having C-2′, C-3′, C-5′, and O-5′ approximately in a plane, with C-4′ displaced above the plane (as viewed when the ring is numbered clockwise). Such a conformation (*a*) brings H-3′ and H-4′ into a quasi-axial arrangement, giving rise to large values of $J_{3',4'}$ and $J_{4',5'}$, (*b*) allows the substituents at C-3′, C-4′, and C-5′ to adopt quasi-equatorial orientations, and (*c*) allows O-2′ to

adopt a quasi-axial orientation for maximum relief from polar destabilization (anomeric effect) while allowing C-1′ to become quasi-equatorial. The coupling data do not establish the favored relative orientations of the different rings in **1**, **2**, or **3**, and no such conformational significance is implied in the representation **3a** for **3**.

Signals of acetyl-methyl protons. — Of the three solvents used in this study, benzene-d_6 gives the greatest spectral dispersion. All eleven acetyl-group signals are observed separately in the spectrum of **2** in benzene-d_6, even at 100 MHz (see Table III). In **1**, two of the eight acetyl-group signals overlapped in the 100-MHz spectrum, and in the spectrum of **3** there were three coincident resonances among the fourteen acetyl groups, even at 230 MHz. A greater degree of signal overlap was observed with spectra measured in chloroform-*d* and in acetone-d_6. In benzene-d_6, some of the acetyl-group signals were highly shielded, as observed with other acetylated sugars[6,8,9].

Although the acetyl-group assignments for the reference compound **4** have been established definitively by synthesis of specifically deuterated derivatives, no such firm assignments have been made for **1**, **2**, and **3**, and suggested assignments made on a comparative basis with data[6] for **4** may not be reliable. The excellent dispersion of the acetate spectral-region observed in the present work with **1**, **2**, and **3** suggests that firm assignment of acetyl-proton signals in **1**, as obtained by study of specifically deuterated derivatives, would provide a useful method for determining the relative reactivities of the different hydroxyl groups in sucrose by the general procedure described[6] for methyl α-D-glucopyranoside.

Signals of the methylene bridges in **2** *and* **3**. — The signals of the methylene-bridge protons in **2** and **3**, in benzene-d_6, chloroform-*d*, and acetone-d_6, are depicted in Fig. 6. Chemical-shift data are given in Table I, and coupling constants are given in Table II. In 1-kestose hendecaacetate (**2**), the two protons of the methylene bridge are slightly nonequivalent in chloroform-*d*, so that, at 220 MHz, an AB quartet

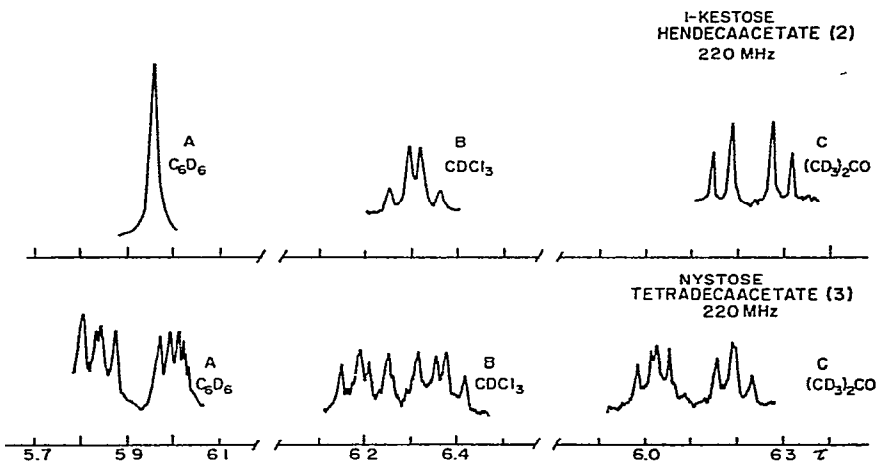


Fig. 6. Signals of the inter-residue methylene bridges of 1-kestose hendecaacetate (**2**) and nystose tetradecaacetate (**3**) in (*A*) benzene-d_6 at 230 MHz, (*B*), chloroform-*d* at 220 MHz, and (*C*) acetone-d_6 at 220 MHz.

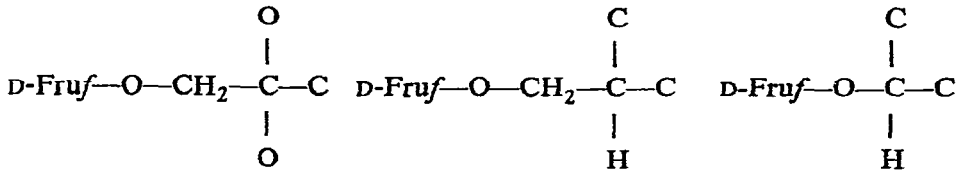
exhibiting a geminal coupling of 9.5 Hz is observed. The nonequivalence is more pronounced in acetone-d_6, but, in benzene-d_6, the protons are practically equivalent, so that an apparent singlet is observed. Nonequivalence of methylene protons is of common occurrence when the methylene group is attached to an asymmetrically substituted carbon atom[10].

Two methylene bridges are present in nystose tetradecaacetate (3), and, in each of the three solvents, the methylene protons in each bridge are nonequivalent. In chloroform-*d*, an 8-line pattern due to the four doublets of two different AB quartets is observed, and a similar pattern is noted in the spectrum measured in benzene-d_6. In acetone-d_6, a similar pattern also arises, but near-superposition of two peaks gives rise to an apparent triplet instead of a pair of doublets. The geminal couplings are, in each case, approximately 9.6 Hz, so that pairs of doublets cannot be correlated on the basis of couplings. However, the appearances of the signals indicate that geminal protons give one signal in the lower-field pair of doublets and one in the higher-field pair; a strongly perturbed pattern would have resulted had adjacent signals arisen from spin-coupled protons.

Structural significance of the n.m.r. data. — The foregoing analyses indicate how a combination of multiple-solvent techniques, measurements at high field-strengths, and the use of spin decoupling permits extensive and detailed interpretations of n.m.r. spectra, even for a compound such as 3, containing 70 protons. The recorded data may be of use in structural characterization of other oligosaccharides. The comparative study of the polymer-homologous series **1**, **2**, and **3** provides a basis for the interpretation of n.m.r. spectra of the acetates of inulin and bacterial levans.

There is little reasonable doubt that the linkage sequence accepted for 1-kestose[3] is correct. Nevertheless, given that 1-kestose is an *O*-D-fructofuranosylsucrose, the observation of the "methylene bridge" signals as an AB pattern establishes independently that the *O*-D-fructofuranosyl group is attached to O-1′ of sucrose. Had the *O*-D-fructofuranosyl group been attached at one of the secondary hydroxyl groups of sucrose, all four methylene groups would have been attached to an acetoxy group (C—CH_2OAc), and no signals in the "methylene bridge" region would have been observed (see Scheme II). Had the *O*-D-fructofuranosyl group been attached to O-6 or O-6′ of sucrose, the signals of a methylene bridge would have been observed because the 6 (or 6′) methylene group would be shielded at least as strongly as those of the 1′ methylene group in the accepted structure **2**. However, attachment through O-6 or O-6′ (see Scheme II) would give a methylene group having a vicinal proton (H-5 or H-5′), so that the signals of the methylene bridge would be observed as the AB part of an ABX system, and not as a simple AB pattern. The AB pattern observed in the "methylene bridge" region serves, therefore, to identify, uniquely, the linkage position of the terminal D-fructofuranosyl group in 1-kestose.

Nystose is an *O*-D-fructofuranosyl derivative of 1-kestose, and the chemical and enzymic evidence so far advanced does not prove the point of attachment of the terminal D-fructofuranosyl group; eleven different positions are available for the linkage, although the $1'_k$ position is considered the most probable point of attachment.



Linkage through O-1′ | Linkage through O-6 or O-6′ | Linkage through a secondary hydroxyl group

Scheme II

The fact that signals of *two* methylene bridges are observed in the spectrum of **3** establishes that the linkage is not through any one of the seven secondary hydroxyl groups of 1-kestose. Furthermore, the fact that the methylene-bridge signals are observed as a pair of AB patterns establishes that the terminal *O*-D-fructofuranosyl group is not attached to O-6, O-$6'_s$ or O-$6'_k$ of 1-kestose, because, in such a situation, one of the methylene bridges would have given a more complex pattern through coupling with H-5, H-$5'_s$, or H-$5'_k$. The pattern of signals observed for the methylene bridges in **3** provides direct verification, therefore, for the linkage pattern previously suggested[4] for nystose.

EXPERIMENTAL

Procedures. — Column chromatography was performed with an acetone-washed mixture of 5 parts (by wt.) of a hydrated magnesium acid silicate, Magnesol (manufactured by the Westvaco Chemical Division of the Food Machinery and Chemical Corp., South Charleston, West Virginia), with 1 part of Celite No. 535 (produced by the Johns-Manville Corp., New York, New York). The chromatograms were developed with 25:1 (v/v) benzene–2-methyl-2-propanol. Zones were detected with a 1% solution of potassium permanganate in 10% sodium hydroxide. Components were recovered from the adsorbent by use of acetone. T.l.c. was performed[11] with silica gel, ~100 μm thick, on polyester sheets (Eastman Kodak Co., Rochester, N. Y.), with 25:1 (v/v) benzene–isopropyl alcohol as the developer and 1-naphthol–phosphoric acid as the indicator. N.m.r. spectra were measured at room temperature with a Varian HA-100 n.m.r. spectrometer, with tetramethylsilane ($\tau = 10.00$) as the internal standard. N.m.r. spectra at 220 MHz were measured with a Varian HR-220 spectrometer equipped with a superconducting solenoid (compare F. A. Nelson and H. E. Weaver, "High Resolution Superconducting Spectrometer", presented at the International Conference on Magnetic Resonance and Relaxation, XIVth Colloque Ampère, Ljubljana, Yugoslavia, September 9th, 1966).

Materials. — *A. Sucrose octaacetate* (**1**). The commercial material (Matheson Coleman & Bell, Norwood, Ohio) was recrystallized twice from 95% ethanol; m.p. 85–86°, $[\alpha]_D^{20}$ +60° (*c* 4, chloroform) [lit.[12] m.p. 89°, $[\alpha]_D^{20}$ +59.6° (chloroform)].

B. 1-Kestose hendecaacetate (2). A mixture of twice recrystallized 1-kestose[3] (500 mg), powdered, fused sodium acetate (500 mg), and acetic anhydride (5 ml) was heated at 80–90° until dissolution was complete (~2 h), and for an additional 2 h. The solvent was removed under diminished pressure at the same temperature, chloroform (15 ml) was added to the residue, and the mixture was washed with saturated, aqueous sodium hydrogen carbonate at 10° until all of the acetic anhydride had been removed. The chloroform solution was dried (sodium sulfate), and evaporated to give **2** as a chromatographically homogeneous syrup; yield 850 mg 88.7%); $R_{sucrose\ octaacetate}$ 0.58–0.59. To ensure the highest degree of homogeneity, the product was purified by column chromatography[13], to give the pure acetate **2** as a colorless syrup, $[\alpha]_D^{20}$ +31.8 ±0.3° (*c* 3.7, chloroform).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.69; H, 5.90.

C. Nystose tetradecaacetate (3). Acetylation of five-times recrystallized nystose[4] (304 mg) by the procedure used for **2**, followed by column-chromatographic purification, gave the acetate **3** as a chromatographically homogeneous syrup, yield 500 mg (87.2%), $[\alpha]_D^{20}$ +19.1 ±0.3° (*c* 2.0, chloroform); $R_{sucrose\ octaacetate}$ 0.33–0.34.

Anal. Calc. for $C_{52}H_{70}O_{35}$: C, 49.76; H, 5.62. Found: C, 49.90; H, 5.89.

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