Note

Physical studies on oligosaccharides related to sucrose. Part III. C.m.r. studies on 1-kestose and nystose

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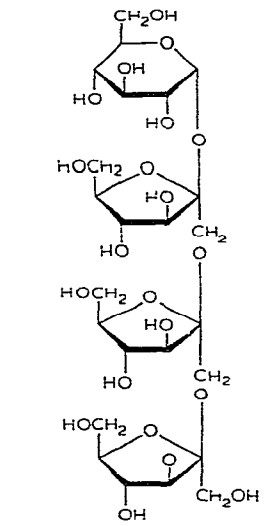
In continuation of a series of papers[1,2] concerned with the characterization, by physical methods, of oligosaccharides related to sucrose, we now describe a study by ^{13}C nuclear magnetic resonance (c.m.r.) spectroscopy of nystose (**1**) and 1-kestose (**2**). Previous reports have concerned the application of high-resolution, p.m.r. spectroscopy[1] and high-resolution, electron-impact ionization mass-spectrometry[2] in investigations of appropriately derivatized members of this series of sugars.

Although inherent, experimental difficulties have, until recently, prohibited the use of c.m.r. spectroscopy as a general method of studying molecules in solution, this technique has now emerged as a potent tool for the investigation of carbohydrates and their derivatives. Whereas several authors have reported fragmentary[3,4] or complete[5–9] c.m.r. spectra of monosaccharides and monosaccharide derivatives, c.m.r. spectral investigations of oligosaccharides have been less common[9–11].

Various workers have tentatively identified the signals in c.m.r. spectra of aqueous solutions of sucrose[10] (**3**), β-D-fructofuranose[9] (**4**), and α-D-glucopyranose[6,7] (**5**), and have arrived at the general conclusion that the ^{13}C nuclei at the anomeric center resonate at lower field than hydroxymethylene carbon nuclei, which, in turn, give signals downfield of the resonance position of ^{13}C nuclei in hydroxymethyl groups.

The c.m.r. spectra of **1** and **2** in methyl sulfoxide-d_6 are shown in Fig. 1. Table I records chemical-shift data measured for solutions of **1** and **2** in methyl sulfoxide-d_6, together with values reported[6,9,10] for aqueous solutions of **3**, **4**, and **5**, referenced to the ^{13}C resonance of internal tetramethylsilane. A similar comparison of $\delta^{c}_{Me_4Si}$ values measured for solutions of stachyose (**6**), raffinose (**7**), and planteose (**8**) in methyl sulfoxide, together with reported[10] values determined for aqueous solutions



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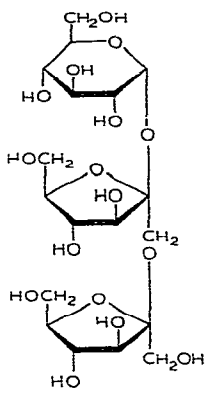

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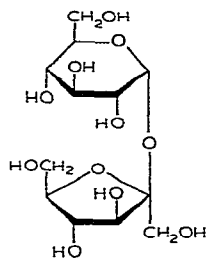

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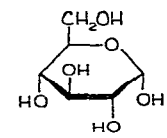

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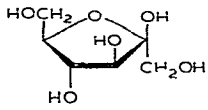

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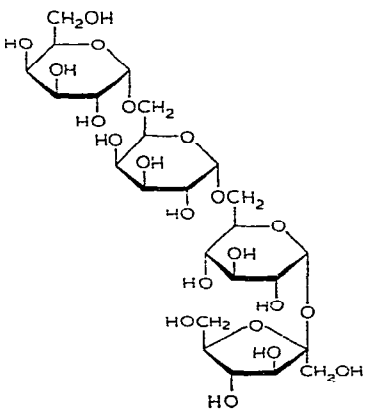

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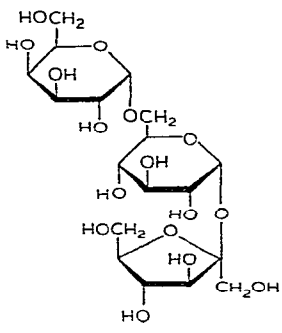

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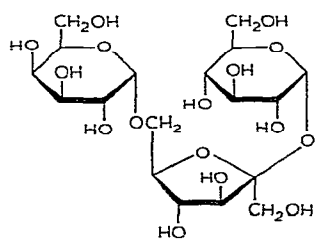

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of the same compounds, is presented in Table II. No systematic change is observed between resonance positions of corresponding nuclei for solutions in water or in methyl sulfoxide*, except for the hydroxymethyl ($-^{13}CH_2O-$) groups, which appear to be shifted slihgtly to higher field in methyl sulfoxide, possibly as a consequence of differences in coordination, at the terminal hydroxyl group, with the solvent. By and large, however, the effects of temperature and solvent seem minor in all of these examples.

The data presented in Table I indicate that the c.m.r. spectrum of nystose (**1**) is very nearly identical with that of the sum of the spectra of 1-kestose (**2**) and β-D-fructo-

*The observed differences of ~1.5 p.p.m. between the δ^c values measured in this study and the corresponding literature values probably result from the approximations implicit in converting, from one standard, data referenced to another standard (see Experimental section).

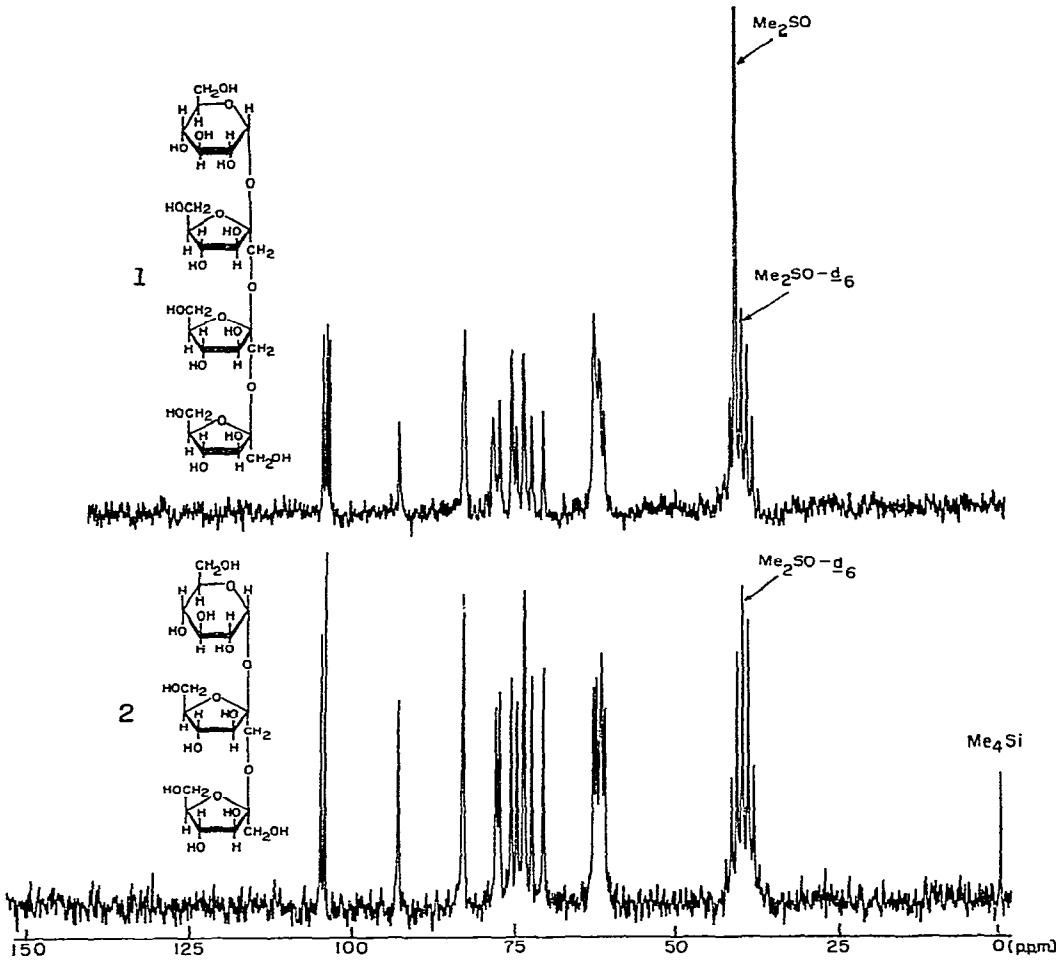

Fig. 1. Fourier-transform, c.m.r. spectra of nystose (**1**) and 1-kestose (**2**), measured at 25.15 MHz in methyl sulfoxide.

furanose (**4**). Similarly, the spectrum of **2** bears a considerable resemblance to the sum of the spectra of sucrose (**3**) and **4**. The relation of the spectrum of **3** to the sum of the spectra of its monosaccharide components (**4** and **5**) is also evident from this Table. It may thus be inferred with some confidence that homologation of D-fructofuranosyl residues in passing from **5**→**3**→**2**→**1** does not produce a major alteration in the shielding interactions present in the D-fructofuranosyl residue.

Anomeric-carbon signals are more clearly differentiated at lower field (<92 p.p.m.) than the other signals, with the C-2 resonances of the ketofuranosyl residues lying at extreme lowest field (103–106 p.p.m.). At the high-field end of the spectrum (>65 p.p.m.), the signals of the carbon atoms of primary hydroxymethyl groups can be distinguished. Positive, exact identification of every resonance in the spectra of **1** and **2** is at present out of the question. The tentative assignments given in Table I

TABLE I

C.M.R. CHEMICAL SHIFTS OF NYSTOSE (1), 1-KESTOSE (2), SUCROSE (3), β-D-FRUCTOFURANOSE (4), AND α-D-GLUCOPYRANOSE (5)[a]

Assignment[b]	*Chemical shift of* ^{13}C *resonances of*				
	1[c]	2[c]	3[d,e]	4[d,f]	5[d,f,g]
Fructose C-2	103.7	103.7	103.4	101.3	
C-2′	103.2	103.2			
C-2″	102.8				
Glucose C-1	92.0	91.9	91.8		92.0
Fructose C-3 or C-4	82.1	82.9	81.1	80.4	
C-3′ or C-4′	81.9	82.9			
C-3″ or C-4″	81.9				
C-4 or C-3	77.7	76.9	76.4	75.4	
C-4′ or C-3′	77.5				
C-4″ or C-3″	76.5	76.4			
C-5	74.6	74.7	73.9	74.4	
C-5″	74.6				
Glucose C-2	74.0	73.8	72.5		72.8
Fructose C-5′	72.8	72.8			
Glucose C-3 or C-5	72.8	72.7	72.1		71.5
C-5 or C-3	71.6	71.5	70.9		71.3
C-4	69.8	69.7	69.1		69.6
Glucose C-6 and fructose C-1 and C-6	61.9	62.0	62.2	62.8	
	61.9	61.6	61.4	62.2	
	61.7				
	61.2	61.0			
	61.1	60.9	60.1		60.8
	61.0				
	60.5	60.3			

[a]Chemical shifts are expressed in p.p.m. downfield of the ^{13}C resonance of tetramethylsilane. [b]Except for the general anomeric-carbon and primary CH_2OH carbon assignments, these are tentative; the assignment of primes to numbers is intended mainly to differentiate between resonances of nuclei occupying similar positions in different D-fructofuranosyl residues of **1** or **2**, rather than to provide a specific identification. [c]Measured in methyl sulfoxide-d_6. [d]Measured in water, [e]Data from ref. 10. [f]Data from ref. 9. [g]Data from ref. 6.

for **1** and **2** are based upon earlier interpretations[6,7,9,10] of the spectra of **3**–**5**; in this Table, primes indicate (probable) increasing distance from the D-glucopyranosyl residue, although the identification of resonances within a set is arbitrary and we anticipate that revision will be needed after more sophisticated methods of analysis have led to definitive identification of each signal.

EXPERIMENTAL

Proton-noise-decoupled, c.m.r. spectra of **1**, **2**, and stachyose (**6**) in 12-mm (outside diameter), thick-walled, sample tubes were recorded with a Varian XL-100-15

TABLE II

C.M.R. CHEMICAL SHIFTS[a] OF STACHYOSE (6), RAFFINOSE (7), AND PLANTEOSE (8)

Chemical shifts of				
Stachyose (6)		*Raffinose* (7)		*Planteose* (8)
103.8[b]	103.6[c]	101.2[b]	103.6[c]	101.5[b]
98.6	98.2	96.7	98.3	96.8
98.6	97.9	89.3	91.8	89.4
91.5	91.9	79.9	81.1	77.6
82.2	81.1	75.1	76.7	74.9
76.9	76.7	72.2	74.1	73.4
74.1	74.1	70.5	72.7	70.8
72.7	72.7	69.1	71.2	70.2
71.2	71.1	68.9	70.9	69.3
71.0	70.9	68.5	70.7	68.6
70.9	70.8	68.0	69.4	68.0
70.0	69.5	67.2	69.4	67.3
69.5	69.5	66.5	69.1	66.6
69.1	69.4	66.2	68.4	66.6
68.7	69.2	64.5	66.0	66.2
68.6	69.2	60.0	62.3	60.0
68.5	68.6	59.6	61.7	58.6
68.3	68.3	58.3	61.0	58.3
68.3	68.2			
66.3	66.3			
66.3	65.9			
62.0	62.3			
62.0	61.7			
60.4	61.0			

[a]In p.p.m. downfield from tetramethylsilane. [b]In methyl sulfoxide. [c]In water; data from ref. 10.

n.m.r. spectrometer fitted with a S-124XL/VFT-100X Fourier-transform accessory; spectra were recorded at 30° by using the deuterium resonance of methyl sulfoxide-d_6 as the lock signal, and they are referenced to internal tetramethylsilane. These spectra represent transforms of data from free-induction decay (0.8 sec, 8192 data points) in a 5-kHz window resulting from application of a 60-μsec pulse to ~20% solutions of **1**, **2**, and **6** in methyl sulfoxide-d_6. Chemical-shift data for **1** and **2** were measured directly from the respective spectra and are expressed as $\delta^c_{Me_4Si} = 192.5 - \delta^c_{CS_2}$. The data presented in Table II were measured from spectra recorded with a Varian HA-100-12 n.m.r. spectrometer that incorporated conventional modifications[7] to allow detection of broad-band, proton-noise-decoupled, c.m.r. spectra in 8-mm sample-tubes at 25.15 MHz. The instrumental sensitivity to the weak signals of these latter samples was enhanced by spectral accumulation (50–100 scans) on a Varian C-1024 computer of average transients. The ^{13}C signal of methyl sulfoxide was employed as the internal calibrant and lock signal; shifts measured from these spectra were converted into $\delta^c_{Me_4Si}$ by means of the equation: $\delta^c_{Me_4Si} = \delta^c_{Me_2SO} - 40.0$.

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