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Proton and carbon NMR chemical-shift assignments for [β-D-Fru*f*-(2 → 1)]$_3$-(2 ↔ 1)-α-D-Glc*p* (nystose) and [β-D-Fru*f*-(2 → 1)]$_4$-(2 ↔ 1)-α-D-Glc*p* (1,1,1-kestopentaose *) from two-dimensional NMR spectral measurements †

Jianhua Liu [a], Andrew L. Waterhouse [a,‡] and N. Jerry Chatterton [b]

[a] *Department of Viticulture and Enology, University of California, Davis, CA 95616 (USA)*
[b] *USDA – Agricultural Research Service, Forage and Range Research, Utah State University, Logan, UT 84322 (USA)*

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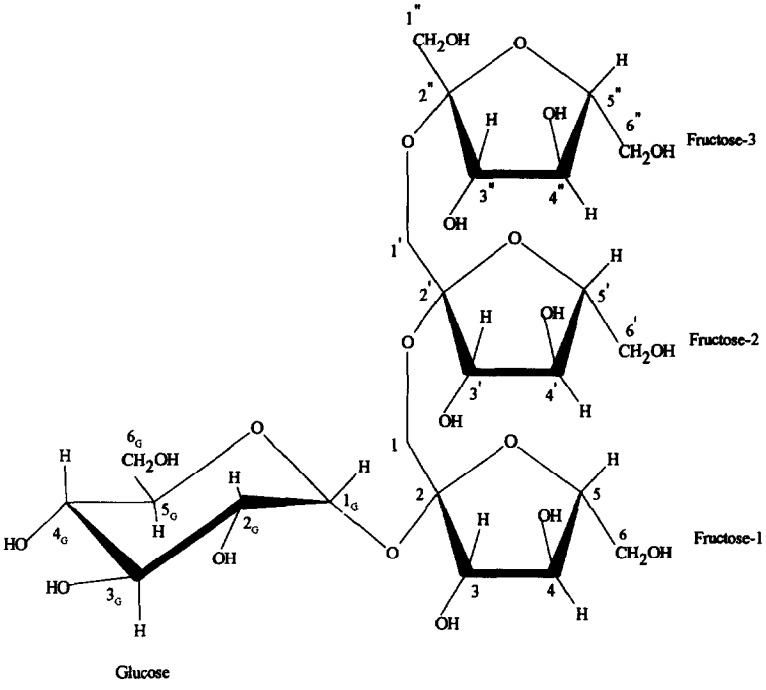
ABSTRACT

The proton chemical-shift assignment of nystose (1) [β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 ↔ 1)-α-D-glucopyranoside], was determined by using two-dimensional (2D) NMR spectral methods, and corrections of, and additions to the previous ^{13}C chemical-shift assignments were made. The ^1H peak of H-1 of the D-glucosyl group was determined by its chemical shift. Signals from fructose-1 were distinguished by the observation of long-range C–H coupling between H-1 of the D-glucosyl group and C-2 of fructose-1. The distinction between fructose-2 and fructose-3 was made by the different $^1J_{CH}$ coupling patterns between C-1 and H-1. Assignments of ^{13}C and ^1H chemical shifts of the related dp 5 compound, β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 ↔ 1)-α-D-glucopyranoside (1,1,1-kestopentaose, 2) are also reported here with comparisons of its spectral data with the data from 1-kestose, nystose and inulin. Based on differences in ^{13}C chemical shifts, it appears that the chemical environment of inulin is not attained in nystose, and only partially attained in 1,1,1-kestopentaose.

INTRODUCTION

Inulin is an important group of compounds classified as fructans that act as storage carbohydrates in numerous plants[1]. Its structure features (2 → 1)-linked β-D-fructofuranosyl units typically terminated by a (2 ↔ 1)-linked α-D-glucopyranoside unit. A special structural feature of inulin is that the fructose rings are not involved in the backbone of the polymer. This has important implications in conformational structure. In particular, one would expect that ring distortions would not interact strongly with linkage conformation. Understanding the spatial structure of inulin may provide information on its physico chemical properties.

* For an explanation of the trivial name, see ref. 9.
† Paper no. 7 of a series: Conformational Analysis of D-Fructans.
‡ Corresponding author.



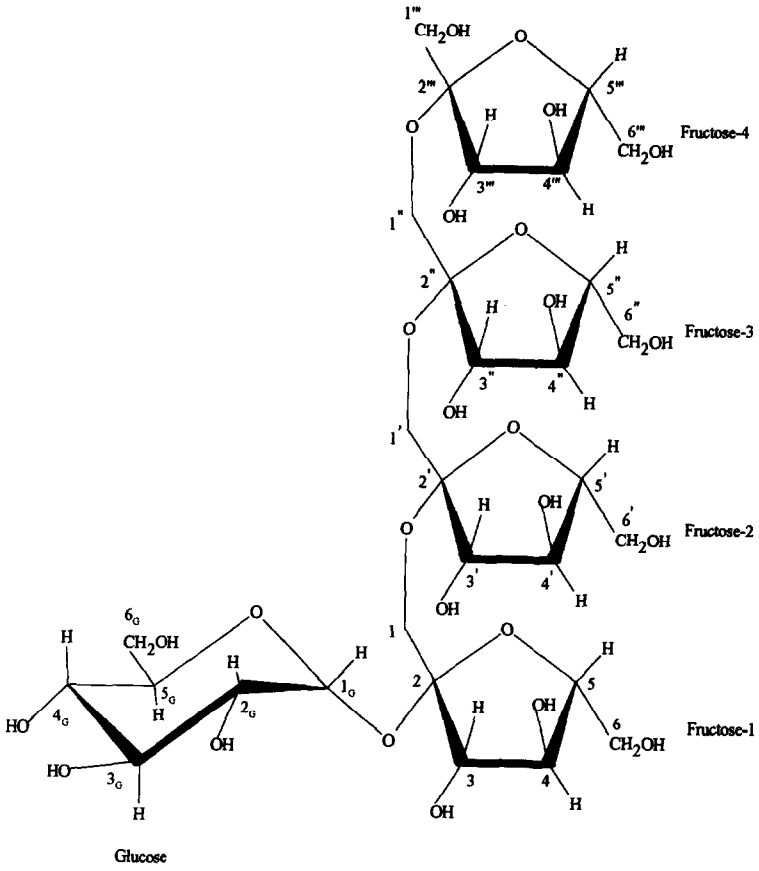
Nystose (1)

While NMR spectroscopy has long been used to identify plant fructans[2–4], until now only ^{13}C spectral assignments of nystose (**1**) have been reported using older NMR methods lacking direct connectivity information, wherein the assignments have either a few errors[5] or are incomplete[6]. Other available data for **1** include ^{1}H signal assignment of nystose peracetates[7] and a recent low-temperature X-ray diffraction study[8,*]. No NMR spectral data has been published for 1,1,1-kestopentaose[9] (**2**). Among the oligosaccharides, complete NMR assignments of all trisaccharides, namely, 1-kestose[10], 6-kestose and neokestose[11], have been reported. Other related structural studies include the ^{13}C NMR assignment of inulin and grass levan[5], the crystalline conformation of inulin by X-ray and electron diffraction study[12] and the determination of molecular configuration of inulin[13].

RESULTS AND DISCUSSION

Chemical-shift assignments of both nystose and 1,1,1-kestopentaose are listed in Table I. In the proton spectra of both compounds, the H-1 signal of the D-glucosyl

* Note added in proof: Another X-ray study of nystose has been described in ref. 22.



1,1,1-Kestopentaose (2)

group can be easily identified by its chemical shift. Other identifiable signals in the 1D ^{1}H spectra, based on the previous work on 1-kestose, are H-2 and H-4 of the D-glucosyl group as well as the sets of H-3 and H-4 of the D-fructosyl groups.

A. Nystose

COSY spectrum[14].—Due to the fact that almost all proton signals are in a range of ca. 1 ppm, heavy signal–signal and signal–diagonal overlaps in COSY experiment are expected. H-2, H-3, H-4, and H-5 of the D-glucosyl group can be assigned by the COSY spectrum alone. One can also find three sets of couplings which belong to H-3, H-4, and H-5 of each D-fructosyl group. All the H-6's cannot be identified by the COSY spectrum because of signal–diagonal overlap. The H-1's of the D-fructosyl groups also cannot be assigned at this point because they are not directly coupled to any proton.

One-bond heteronuclear (C–H) shift correlation spectrum[15].—This experiment was carried out to correlate proton assignments to carbon assignments. By this

TABLE I

^{13}C and ^{1}H NMR assignments of nystose (**1**) and 1,1,1-kestopentaose (**2**) [a] and the ^{13}C chemical shifts of inulin

^{13}C						^{1}H					
Literature				This Work		Literature		This Work			
1-Kestose[10]		1[5]	Inulin[5]	1	2	1-Kestose[10]		1		2	
1	62.17	62.05		62.32	62.24	3.54	3.66	3.56	3.66	3.58	3.64
1′		62.20	62.2	62.13	62.13 [b]			3.54	3.68	3.57 [c]	3.69
1″	61.70	61.53		61.70	61.90 [b]	3.50	3.59	3.50	3.58	3.56 [c]	3.70
1‴					61.68					3.54	3.62
2	104.50	104.90		104.47	104.46						
2′		104.26	104.4	104.27	104.28						
2″	104.96	104.43		104.90	104.28						
2‴					104.92						
3	77.92	77.93 [d]		78.08	77.99	4.12		4.10		4.10 [e]	
3′		78.70	78.3	78.77	78.77			4.05		4.04	
3″	77.94	77.89 [d]		78.04	78.60	4.02		4.01		4.05	
3‴					77.99					4.01 [e]	
4	75.12	75.05		75.16	75.10	3.88		3.87		3.87	
4′		75.50	75.6	75.73	75.56			3.90		3.88 [f]	
4″	75.74	75.83		75.62	75.56	3.91		3.93		3.89 [f]	
4‴					75.69					3.92	
5	82.46	82.44		82.47	82.48	3.67	3.70	3.70		3.71	
5′		82.30	82.3	82.32	82.33			3.69		3.69	
5″	82.36	82.30		82.32	82.33	3.69	3.70	3.69		3.69	
5‴					82.33					3.69	
6	63.44	63.46		63.53	63.53 [g]	3.63		3.56	3.60	3.69 [h]	3.75
6′		63.46	63.4	63.50	63.45 [g]			3.67	3.69	3.58 [h]	3.64
6″	63.59	63.46		63.44	63.45 [g]	3.64		3.62	3.64	3.55 [h]	3.66
6‴					63.45 [g]					3.69 [h]	3.73
1_G	93.73	93.74	93.7	93.74	93.74	5.26		5.27		5.26	
2_G	72.39	72.40	72.8	72.41	72.43	3.38		3.37		3.37	
3_G	73.85	73.80	74.3	73.86	73.84	3.59		3.59		3.59	
4_G	70.48	70.40	70.6	70.49	70.47	3.30		3.30		3.29	
5_G	73.67	73.65	73.5	73.68	73.69	3.64	3.68	3.67		3.66	
6_G	61.40	61.28	n.a.	61.41	61.36	3.63		3.64		3.64	

[a] Chemical shifts are in ppm with an internal standard of acetone at 2.05 ppm for ^{1}H and 29.80 ppm for ^{13}C. For the meaning of the atom numbers, see the formulae.
[b,c,d,e,f,g,h] Assignments may be interchanged within a given set.

experiment the ^{13}C signals from the D-glucosyl group (except C-6 at this point) and three sets of ^{13}C signals from C-3, C-4, and C-5 of D-fructosyl groups can be easily assigned. For those unassigned signals the ^{1}H and ^{13}C signals are paired so that only one of the paired signals needs to be assigned by another experiment.

One-bond C–H coupling cross peaks between C-1 and H-1 of the D-fructosyl groups showed a clear difference between the ^{13}C signal at 61.70 ppm and the other two at 62.13 and 62.32 ppm. We expect that the signal from H-1 of fructose-3

to be different based on the fact that it is a terminal hydroxyl group instead of being in the linkage backbone. Thus it will have less splitting due to partial equilibration of the chemical environment from rapid rotation, and that is observed.

Long-range heteronuclear (C–H) shift correlation spectrum[16].—In this experiment one-bond C–H couplings were repressed by the bilinear rotational decoupling (BIRD) pulse, so that only long-range couplings were presented.

Coupling between H-4 of the D-glucosyl group to the ^{13}C signal at 61.41 ppm reveals the C-6 assignment. With the information from the COSY and one-bond (C–H) correlation experiments, both ^{1}H and ^{13}C assignments for the D-glucosyl group are completed.

Coupling between H-1 of the D-glucosyl group and C-2 of the D-fructosyl group gives the key to distinguishing fructose-1 from the other two. So the ^{13}C signal at 104.47 ppm is assigned to C-2 of fructose-1. Based on the distinction of fructose-3 by the one-bond C–H correlation experiment, the signal at 104.27 ppm is assigned to fructose-2, and the one at 104.90 is assigned to fructose-3. Starting from the C-2 assignment of fructose-1, the couplings of C-2 to H-1, H-1 to C-3, C-3 to H-4, H-4 to C-5 and C-6, H-3 to C-1 allow the unambiguous carbon and proton signal assignments for fructose-1. Signals from the other two D-fructosyl groups are assigned by virtually the same scheme except for those signals which are not resolved. All ^{13}C and ^{1}H chemical-shift assignments are listed in Table I. Assignments of C-6's of the D-fructosyl units are based on the coupling between C-6 and H-4. Note that in the one-dimensional ^{13}C spectrum of nystose, these signals appears to have exactly the same frequency when a line-broadening factor is applied to the raw data, but by using resolution enhancement they are observed to be separated by 12 and 30 Hz.

Homonuclear (H–H) J-resolved spectrum[17].—This experiment is usually carried out to resolve the multiplicities of the ^{1}H signals. Compared to the same experiments used in the NMR assignments of 1- and 6-kestose, the usefulness of this experiment is hampered by severe signal overlap. One can still use this experiment to find the precise chemical shifts and coupling constants for certain signals, but it is not possible to find all the coupling constants and multiplicities. Some of the ^{1}H–^{1}H coupling constants obtained from this experiment, as well as from the one-dimensional ^{1}H spectrum, are listed in Table II.

B. 1,1,1-Kestopentaose

We have assigned the ^{13}C chemical shift data for 1,1,1-kestopentaose, largely by comparing its chemical shift data with that of nystose and 1-kestose[10]. Our assignment was aided by ^{1}H spin coupling patterns. The differences between ^{13}C signals from C-4's of D-fructosyl units of nystose and 1,1,1-kestopentaose preclude unambiguous assignment of these signals, but information from the COSY spectrum gives clear H-3, H-4 coupling information supporting our assignments. ^{1}H signals can be assigned accordingly by the one-bond heteronuclear (C–H) shift-correlation spectrum except those protons attached to carbons with overlapped ^{13}C signals. These assignments are also listed in Table I.

TABLE II

^1H–^1H coupling constants (Hz) from 1-kestose, nystose and 1,1,1-kestopentaose

Coupling pair	1-Kestose[19]	Nystose		1,1,1-Kestopentaose
	J_{obs}	J_{obs}	J_x [a]	J_{obs}
Fructose-1				
3–4	8.00	8.73	8.48	8.73
4–5	8.00	8.64	8.68	8.44
Fructose-2				
3–4	8.50	8.40	7.54	8.22
4–5	7.50	8.24	9.24	8.22
Fructose-3				
3–4		8.44	9.47	8.36
4–5		8.06	8.82	7.78
Fructose-4				
3–4				8.58
4–5				7.69

[a] Calculated from crystallographic data; see discussion section.

C. Comparison of the NMR spectra of the trimer, tetramer, and pentamer with that of the inulin

All the ^{13}C NMR spectral assignments from this work and from the literature on the trimer[10], tetramer, pentamer, and polymer[5] are tabulated in Table I. One interesting point here is to see when and if the fructans attain a regular helical structure. To observe this, one would look for a single chemical environment for the internal fructosyl units. This would be evident by the appearance of single lines for each fructosyl carbon line, except for the terminal fructosyl groups. End effects would always ensure that the chemical environment of these carbons was different. That is what is seen for the C-2 and C-4 fructosyl signals: the middle fructosyl units carbon signals appear at the same frequency as inulin, and the terminal fructosyl units have unique shifts. The chemical environments of the fructosyl C-5 and C-6 atoms each appear to be very similar, even for the short fructans; however, there is a significant dispersion of the signals for some of the other carbons. The C-1 signals have over 0.5 ppm dispersion for both **1** and **2**, and no coalescence appears to be occurring.

The reason for the disparate behavior of these different signals may be that the cyclic portion of the fructosyl rings in **2** (fructose-2 and -3) are in fact attaining a similar ring form in solution. This hypothesis is also supported by the very similar H-3–H-4 and H-4–H-5 coupling constants observed for these rings. On the other hand, the wide variation in C-1 suggests that the linkage conformation has not attained a regular helical form in **2**, and it is possible that such inulin type linkages do not have a regular helical pattern in solution. This observation emphasizes that when the unit rings are not part of an oligomer's linkage backbone, these two elements of the structure can behave independently.

D. Comparison of the solution and crystal structure of nystose ring forms

The $^1H-^1H$ coupling constants of H-3 to H-4, and H-4 to H-5 of the D-fructosyl units can provide information about ring conformation. To compare the solution and crystal structure of nystose, $^1H-^1H$ coupling constants were measured in D_2O, theoretical values were calculated from crystallographic data, and the results were tabulated in Table II. The solution H-3–H-4 coupling constants are all very similar, varying between 8.0 and 8.7 Hz. One can calculate a coupling constant from a single ring form that would yield a coupling constant of 8.4 Hz, and that is the $^4T_3$ form, using a conformational system developed by Cremer and Pople[18]. Assuming that the coupling observed is the result of largely one conformation and not a mixture of many different interconverting forms, it appears that this is the one form that exists in solution. The X-ray crystal structure of nystose[8] was optimized by MM2(87) with all heavy atoms fixed. The coordinates of hydrogens from such a calculation is generally more accurate than crystallographic data. The resulting structure was used to calculate hypothetical $^1H-^1H$ coupling constants[19]. In the crystal structure, three D-fructosyl rings take different ring forms, namely, $_3E$, $^4T_5$, and $^4T_3$ for fructose-1, fructose-2, and fructose-3, respectively[8]. This case is similar to 1-kestose, where the fructose-1 ring takes the $^3T_4$ form, while the fructose-2 ring takes the $^4T_3$ form in the crystal[20] and both have $^4T_3$ ring forms in solution[21]. This difference in the ring forms of nystose in solution and the crystal may be caused by deformation during the crystallization process. The crystallographic form is reported to have all 14 hydroxyl groups involved in both two- and three-centered intermolecular H-bonds, which could support such deformation.

EXPERIMENTAL

Solutions of **1** and **2** (150 mg) in D_2O were transferred to 5-mm NMR sample tubes. NMR spectra (1D and 2D) were recorded with a Bruker AMX 360 wide-bore spectrometer with an inverse 5-mm broad band probe operating at 360.13 MHz for ^1H and 90.56 MHz for ^{13}C, and a Bruker AM 600 spectrometer operated at 600.13 MHz for ^1H and 150.93 MHz for ^{13}C.

COSY spectra.—These experiments were carried out using the Bruker AM 600 instrument with a spectral width of 2403.846 Hz for both dimensions with 256 experiments and 16 scans for each experiment acquiring 1K complex data points. When doing the Fourier transform, zero filling once in the $F2$ dimension and twice in the $F1$ dimension were performed, and a sine window with a shift of $\pi/3$ radians was used. The total experiment time was about 2 h.

Homonuclear (H–H) J-resolved spectra.—For nystose, the spectrum was recorded using the Bruker AMX 360 instrument at a sweep width of 1865 Hz in the $F2$ dimension and 30 Hz in the $F1$ dimension. Forty experiments and 32 scans for each experiment were performed. The total experiment time was about 30 min. For 1,1,1-kestopentaose, it was performed on the Bruker 600 instrument with a sweep width of 2403.846 Hz in the $F2$ dimension and 50 Hz for the $F1$ dimension,

acquiring 256 experiments with 16 scans for each experiment. The total experiment time was about 3 h.

One-bond C–H correlation spectra.—These experiments were carried out using the Bruker AM 600 spectrometer (for ^{13}C detection and for 1,1,1-kestopentaose only) and the Bruker AMX 360 instrument (for ^{1}H detection).

The ^{13}C detected spectra were comprised of 202 experiments with 160 scans for each experiment using 2K complex data points with a sweep width of 7575.758 Hz in the $F2$ dimension and 2403.846 Hz in the $F1$ dimension. The total experiment time was about 10 h.

The ^{1}H-detected spectrum for nystose was recorded with a sweep width of 1260 Hz in the $F2$ dimension and 6250 Hz in the $F1$ dimension, using 256 experiments with 32 scans per experiment with using composite pulse ^{13}C decoupling. The total experiment time was about 1.5 h.

Long-range C–H correlation spectrum.—This experiment was only performed for nystose on the Bruker AMX 360 spectrometer using ^{1}H detection. The experimental conditions are the same as in the one-bond C–H correlation experiment, except that it was recorded over 128 experiments, with 192 scans per experiment, and the total experiment time was about 10 h.

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