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Proton and carbon chemical-shift assignments for 1-kestose, from two-dimensional n.m.r.-spectral measurements*

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ABSTRACT

The proton chemical-shift assignment of a simple relative of inulin, namely, 1-kestose [*O*-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl α-D-glucopyranoside], was determined by using two-dimensional (2D) homonuclear and heteronuclear n.m.r.-spectral methods, and corrections of, and additions to the ^{13}C-chemical-shift assignments were made. The ^{1}H peak of H-1 of the D-glucosyl group was determined by its chemical shift. Using H–H coupling information for this proton, the chemical shift of most of the proton signals of the β-D-glucosyl group was determined. Although the signals from the two D-fructosyl units were very similar, long-range C–H coupling allowed their complete C and H assignment. In particular, the coupling of the H-1 atom of D-glucose to C-2′ of D-fructose **1** allowed distinction between the two D-fructose units.

INTRODUCTION

1-Kestose (**1**) was first observed[1] during the action of yeast invertase preparations on concentrated solutions of sucrose. Naturally occurring **1** is found in honey[2] and in some plants belonging to the Amaryllidaceae[3] family and the genus *Eryngium*[4]. It is one of three related trisaccharides that differ only by the linkage from the second D-fructosyl group to the sucrose. The two others are 6′-*O*-β-D-fructofuranosylsucrose (6-kestose) and 6-*O*-β-D-fructofuranosylsucrose (neokestose). 1-Kestose is a precursor of inulin, one of the important D-fructans, and the assignment of chemical shifts provides a basis for determining the orientation of the inter-residue linkage in **1**, which is essential for analyzing the structure of related polymers.

In 1969, Binkley and co-workers reported a ^{1}H-n.m.r.-spectral assignment of 1-kestose peracetate[5]. This was among the first n.m.r. studies made on analogs of **1**. To date, only the ^{13}C-n.m.r. spectrum of **1** has been assigned[6,7].

The assignment of the spectra of 1-kestose will allow conformational analysis of the compound. However, interpretation of conformational data for this flexible mole-

* Part I of a series entitled "Conformational Analysis of D-Fructans".

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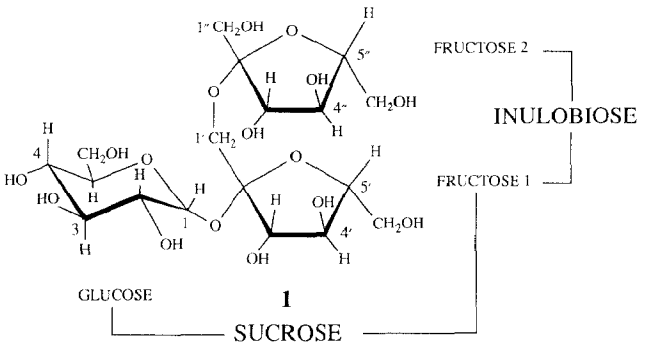

cule will require an analysis of the distribution of the conformer populations as determined by their relative energies.

RESULTS AND DISCUSSION

The 2D n.m.r. shift correlation and J-resolved spectra of **1** are shown in Figs. 1 and 2, and Table I gives the chemical-shift assignments. A key factor in the complete ^1H chemical-shift assignment of **1** was the unambiguous assignment of H-1. Its signal was the only easily identifiable peak in the 1D spectrum.

COSY[8] *and COSY-RCT*[9] *spectra.* — The ^1H signals of H-2, H-3, H-4, and H-5

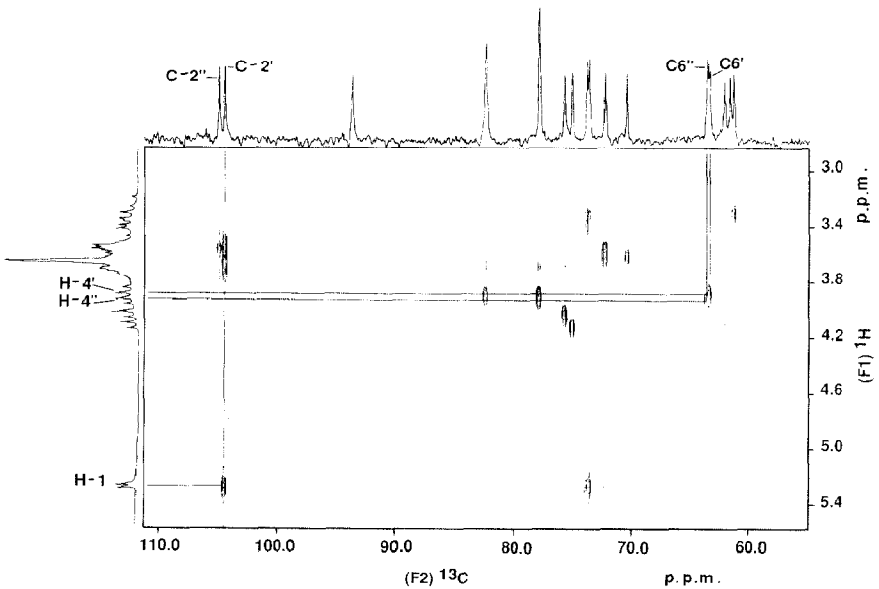

Fig. 1. Long-range heteronuclear (C–H) shift correlation spectrum.

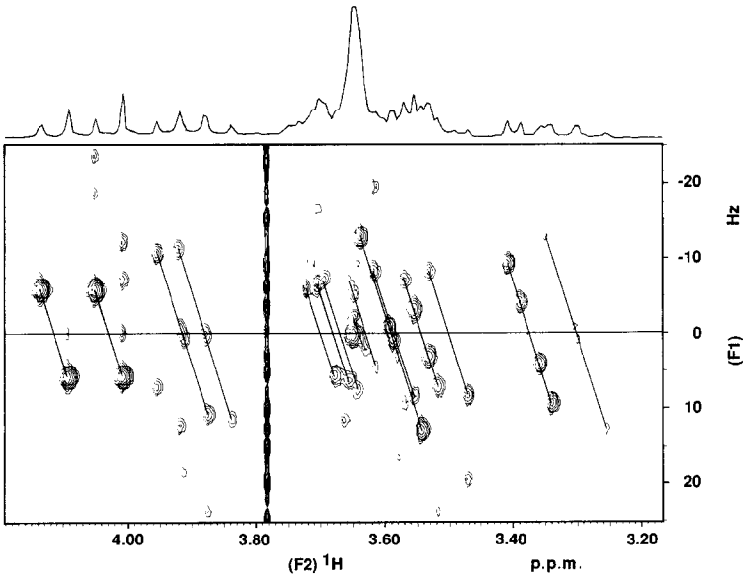

Fig. 2. Homonuclear (H–H) J-resolved spectrum.

were assigned by using the homonuclear shift-correlated 2D n.m.r. (COSY) spectrum of **1**, and these assignments were confirmed by the COSY-RCT (COSY with one step relayed coherence transfer) experiment. The COSY spectrum also provided the connectivity between the 3, 4, 5, and 6 positions of the D-fructosyl units, and the coupling pattern allowed their identification; however, these rings could not be distinguished.

The COSY-RCT spectrum provided convincing evidence that the foregoing assignment of the H-2, H-3, H-4, and H-5 chemical shifts was correct. By detecting relayed couplings in ^{1}H systems, by using a third spin as a mediator to transfer information from one spin to another[10], cross-peaks between separately uncoupled nuclei are observed.

Consequently, in the COSY-RCT spectrum of **1**, cross-peaks between H-1 and H-3 were observed, because both are coupled to H-2. Also, cross-peaks between the H-3 and H-5 atoms and between the H-4 and H-6 atoms of the two D-fructosyl units were observed, substantiating the previous assignments.

One-bond heteronuclear[11] *(C–H) shift correlation spectrum.* — This experiment was carried out in order to correlate the aforementioned ^{1}H assignments with the literature ^{13}C assignments[6,7]. Data obtained from the experiment showed distinctly separated C-4′–H-4′ and C-4″–H4″ cross-peaks, thus confirming the assignments made for H-4′ and H-4″, that is, assuming that the reference ^{13}C chemical-shift assignment was accurate. These results, combined with the results from the COSY and COSY-RCT experiments, also confirmed the assignments for H-3′, H-3″, H-5′, H-5″, H-6′, and H-6″.

In the previous ^{13}C-assignment studies which were carried out on analogs, it was

TABLE I

N.m.r. data for 1-kestose

Atom number	*^{13}C Shift[a] (p.p.m.)*	*^{1}H Shift[b] (p.p.m.)*	*Assigned ^{1}H*	
			COSY/COSY-RCT cross-peaks	*Long-range C–H corrected cross-peaks*
1	93.73	5.26	2, 3	
2	72.39	3.38	1, 3, 4	1, 3
3	73.85	3.59	2, 4, 5	1, 2, 4
4	70.48	3.30	3, 5	2, 3
5	73.67	3.64	3, 4	1, 4, 6
		3.68		
6	61.40	3.63	5	4
1′	62.17	3.54		3′
		3.66		
2′	104.50[c]			1, 1′, 4′, 5′
3′	77.92[c]	4.12	4′, 5′	4′
4′	75.12	3.88	3′, 5′	3′, 6′
5′	82.46[c]	3.67	3′, 4′, 6′	3′, 4′
		3.70		
6′	63.44[c]	3.63	4′	4′, 5′
1″	61.70	3.50		3″
		3.59		
2″	104.96[c]			1″, 5″
3″	77.94[c]	4.02	4″, 5″	4″
4″	75.74	3.91	3″, 5″, 6″	3″, 5″
5″	82.36[c]	3.69	3″, 4″	4″
		3.70		
6″	63.59[c]	3.64	4″	4″

[a] Measured from acetone as internal standard, at 31.48 p.p.m. [b] Measured from acetone as internal standard, at 2.06 p.p.m. [c] New ^{13}C chemical-shift assignment.

not possible to distinguish between C-5′ and C-5″, and in this study at 50 MHz these signals were not resolved until some resolution enhancement was applied to the data. However, these signals could be assigned from the H-5′–C-5′ and H-5″–C-5″ cross-peaks, and the chemical shift of C-5″ is downfield of C-5′ by 10 Hz.

On the other hand, the C-3′ and C-3″ ^{13}C peaks were not resolvable at 50 MHz because their line widths were greater than their separation. However, the H-3′–C-3′ cross-peak was distinctly separated from the H-3″-C-3″ cross-peak due to ^{1}H shift differences, and slices of these cross-peaks taken from the ^{13}C (F2) dimension showed that the C-3″ signal was downfield that of the C-3′ signal by ~ 1 Hz. Cross-peaks from the H-1′–C-1′ and H-1″ C-1″ couplings indicated that the H-1′ signal was downfield to that of the H-1″ signal.

Long-range heteronuclear (C–H) shift correlation spectrum. — The bilinear rotational decoupling (BIRD) pulse developed by Martin and co-workers[12] to repress 1-bond C–H coupling revealed only long-range couplings.

Several details could be noted from the long-range (two- and three-bond), C–H shift correlation spectrum (see Fig.1). First, the chemical shift of C-2′ was corrected. An earlier ^{13}C assignment[6,7] of C-2′ placed it downfield of C-2″, whereas data from the long-range, C–H shift-correlation spectrum proved otherwise. In the spectrum, H-1 showed a cross-peak with an upfield peak at 104.50 p.p.m. in the ^{13}C spectrum, indicating this to be the signal from C-2′, not from C-2″ (as earlier reported). The proximity of H-1 and C-2′ made it possible for coupling to occur, but coupling between H-1 and C-2″ is not observable, because C-2″ is 6 bonds away from H-1. The correct assignment of C-2′ then led to further evidence which substantiated the preliminary assignments made on the D-fructosyl units. The C-2′–H-4′ cross-peak showed that these two atoms are part of the same ring-structure, confirming the literature assignment of the other ring-carbon atoms of the D-fructose units at the same time.

Based on the H-6–C-4 cross-peak, the H-6 signal was also assigned. The H-6 signal had not been identified in the previous 2D experiments because the cross-peaks were either obscured by other cross-peaks or the cross-peaks coincided with other cross-peaks when projected to the F1 dimension, thereby making the assignment difficult.

The long-range C–H shift correlation spectrum also gave H-4′–C-6′ and H-4″–C-6″ cross-peaks. Jarrel and co-workers[7], in 1979, were unable to distinguish between the C-6′ and C-6″ signals. Results obtained from the long-range, C–H shift-correlation spectrum assigned these two signals. Although very close to each other, their coupling with H-4′ and H-4″ indicated that the signal of C-6′ is slightly upfield of that of C-6″.

Selective heteronuclear (C–H) J-resolved spectrum. — By using a "fake" Gaussian selective ^{1}H 180-degree pulse, a new C–H, J-resolved, 2D experiment was created from a literature[13] experiment. The J dimension (F1) shows coupling with only the ^{1}H that was flipped by selective irradiation; there was splitting for all nuclei coupled to the irradiated proton, and there were singlet ^{13}C peaks for all C nuclei not coupled to the irradiated proton. This allowed further discrimination of the signals from the two D-fructosyl units. Some ^{13}C signals from D-fructose 1 show splitting, being just three or four bonds away. ^{13}C-N.m.r. signals from D-fructose 2, being more than five bonds away, did not show any splitting. The $^{3}J_{C\text{-}H}$ value for the coupling between H-1 and C-2′ obtained from the experiment was 4.28 Hz. This information is vital in determining the orientation of the inter-residue linkage of the sucrose moiety in **1**.

Homonuclear (H–H) J-resolved spectrum. — The determination of the multiplicities of the ^{1}H signals were oftentimes complicated by overlapping or unresolved peaks, but the problem was overcome by employing the homonuclear, J-resolved experiment[14]. The experiment disperses the coupling pattern in the F1 dimension enabling not only the determination of signal multiplicity, but also of coupling constants and precise chemical shifts, even for overlapped signals. The splitting patterns of the H-5 signals of all the sugar residues in **1**, for example, were completely indiscernible in the 1D ^{1}H spectrum but were determined from the J-resolved spectrum (see Fig. 2). The coupling patterns facilitated the assignments where signals were too close to be discriminated between from correlation spectra.

The existence of multiple crosspeaks for the H-5 signals in some correlation experiments, and the existence of some weak extra peaks in this J-resolved spectrum may point to the existence of two conformers in D_2O solution, although the exchange rate that would be expected should preclude multiple signals. This experiment is also susceptible to artifacts in non-first-order signals, and this may be an example of that problem.

EXPERIMENTAL

A solution of **1** (400 mg) in D_2O (2.5 mL) was transferred to a 10-mm n.m.r. sample-tube. Nuclear magnetic resonance spectra, 1D and 2D, were recorded with a Bruker AF-200, narrow-bore spectrometer with a broad-band probe operated at 200.13 MHz for ^1H and 50.32 MHz for ^{13}C. All of the shift correlation spectra were recorded using a sweep width of 2950 Hz for the ^{13}C dimension and 550 Hz for the ^1H dimension. The homonuclear (H–H) J-resolved spectrum was obtained at a sweep width of 537.6 Hz in the F2 dimension and 33.6 Hz in the F1 dimension. The heteronuclear (C–H) J-resolved spectrum was recorded using a sweep width of 2840 Hz in the F2 dimension and 25 Hz in the F1 dimension.

The number of scans and number of experiments for each spectrum varied, depending on the sensivity of the method used and the availability of time. The COSY and COSY-RCT spectra were obtained by acquiring 128 experiments of 32 scans, using 1000 complex data points. The total experimental time for each was about 4.5 h. A water suppression routine, by T2-relaxation, was incorporated into the COSY experiment in order to eliminate the water peak. Both of the C–H shift correlation spectra were comprised of 80 experiments, each having 2000 complex data points. The one-bond C–H correlation spectrum was recorded using 352 scans per experiment, requiring a total experimental time of 16 h, while the long-range C–H correlation experiment was recorded using 400 scans, and it required a total experimental time of 18 h.

A total of $\sim$1 h was needed for the homonuclear (H–H) J-resolved experiment. The spectrum was recorded using 2000 complex data points and by acquiring 64 experiments of 16 scans each. The heteronuclear (C–H) J-resolved experiment took 12 h and was obtained using 1000 complex data points and 80 experiments of 200 scans each.

The heteronuclear, J-resolved experiment was a modified version of a literature[13] experiment. The 180-degree ^1H low-power, selective pulse was replaced by a "fake", Gaussian, 180-degree, selective pulse which consisted of 33 individual pulses separated by 1-ms delays. The length of each successive pulse was calculated to rise and fall in a Gaussian manner, and the total pulse-length equalled a 180-degree pulse (which was determined in a previous experiment to be 66 ms).

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