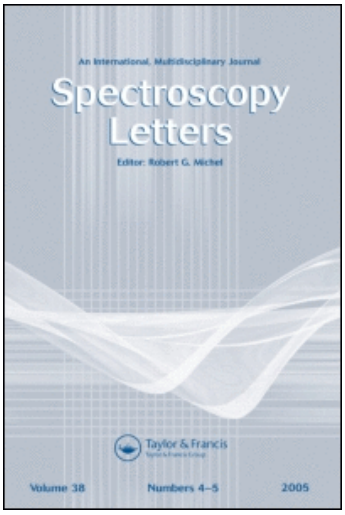

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

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Online publication date: 10 August 2002

To cite this Article Best, Robert B. , Jackson, Graham E. and Naidoo, Kevin J.(2002) 'AN NMR INVESTIGATION INTO THE DYNAMICS OF PANOSE, AN α(1→4) AND α(1→6)-LINKED TRISACCHARIDE', Spectroscopy Letters, 35: 5, 625 — 632
To link to this Article: DOI: 10.1081/SL-120014935
URL: http://dx.doi.org/10.1081/SL-120014935

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SPECTROSCOPY LETTERS
Vol. 35, No. 5, pp. 625–632, 2002

AN NMR INVESTIGATION INTO THE DYNAMICS OF PANOSE, AN α(1→4) AND α(1→6)-LINKED TRISACCHARIDE

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ABSTRACT

A complete ^{1}H and ^{13}C assignment of panose, α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→4)-α,β-D-glucopy-ranose, is reported together with the ^{13}C relaxation times measured at 200, 300 and 400 MHz. These data have been used to calculate the order parameters of the sugar rings. The results indicate that the central residue has less motional freedom than the terminal residues. In addition, the motion of residue 1 C6 is also severely restricted. This is explained in terms of hydrogen bonding between O6 of residue 1 and O1 and O2 of residue 3.

Key Words: Panose; Trisaccharide; Starch; NMR; Dynamics

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DOI: 10.1081/SL-120014935 0038-7010 (Print); 1532-2289 (Online)

INTRODUCTION

Starch, a major plant storage polysaccharide, occurs in granules of up to 100 μm diameter. It is actually a mixture of two polysaccharides, amylose, a linear α(1→4)-linked glucose polymer and amylopectin, which consists of short α(1→4)-linked chains with α(1→6)-linked branches approximately every 30 residues.[1,2] The two constituents have different physical properties, the amylopectin being more water soluble.[3] This property is important as it has been proposed that starch or a modified variant of starch could be used as a biodegradable substitute for moulded plastic. Such packaging material and eating utensils have already been made, however, the their water resistance needs to be improved.[4]

The different physical behaviour of amylose and amylopectin has been attributed to the different flexibility of the α(1→4) and α(1→6) glucose linkages. In order to investigate this further, we have studied the solution structure and dynamics of maltose, an α(1→4)-linked disaccharide, and isomaltose an α(1→6)-linked disaccharide.[5] In this paper, we extend the study to panose (α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→4)-α,β-D-glucopyranose), which is the simplest compound containing both the (1→4) and (1→6) linkages. The molecule is shown in Fig. 1, with our residue numbering indicated.

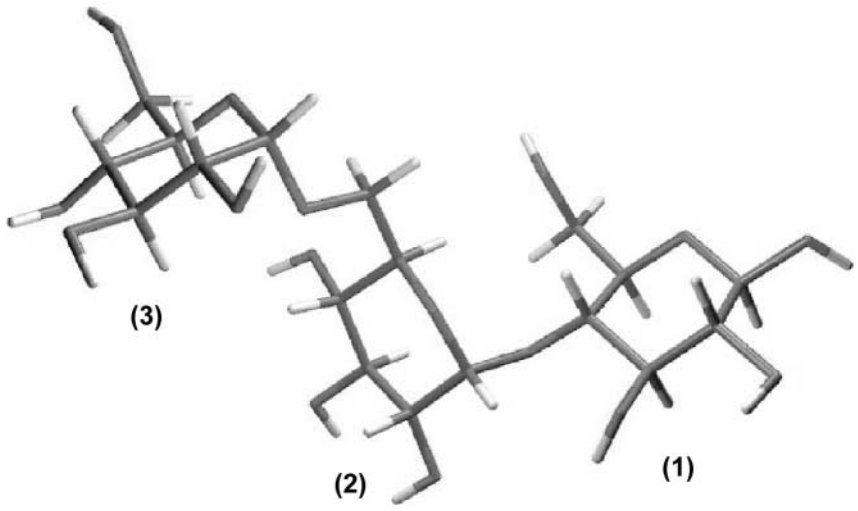

Figure 1. Structure of panose, indicating the residue numbering used. Standard glucose numbering was used for the carbons and protons.

Theory

In carbohydrates, the relaxation of ^{13}C nuclei is due, primarily, to the dipolar interaction with the directly attached proton, if the contribution of chemical shift anisotropy may be neglected.[6] When this is the case, it has been shown that T_1 is given by Eqs. 1.1 and 1.2.

$$T_1^{-1} = \frac{1}{4} D^2 [J(\omega_H - \omega_X) + 3J(\omega_X) + 6J(\omega_H + \omega_X)] \tag{1.1}$$

$$D = \gamma_X \gamma_H \hbar \langle r_{XH}^3 \rangle^{-1} \tag{1.2}$$

The magnetogyric ratios of the carbon and its attached proton are denoted by γ_X and γ_H respectively, and r_{XH} is their internuclear distance. $J(\omega)$, the spectral density describing the motion of the carbohydrate is determined by the motion of the unit vector along the C-H bond in the laboratory coordinate frame, μ_{LF}, via Eqs. 2.1 and 2.2, where $P_2(x)$ is the second Legendre polynomial.

$$J(\omega) = 2 \int_0^\infty C(t) \cos(\omega t) dt \tag{2.1}$$

$$C(t) = \frac{1}{5} \langle P_2(\mu_{LF}(0) \cdot \mu_{LF}(t)) \rangle \tag{2.2}$$

Hence, from nmr relaxation measurements, it is possible to obtain the spectral density directly. However, it is not possible to extract the detailed, atomistic, dynamics (i.e. the motion of μ_{LF}) from the spectral density. Lipari and Szabo[7,8] have developed a model free formalism for the interpretation of relaxation data. In this approach the motional correlation function $C(t)$ is formally factored into molecular tumbling, $C_O(t)$, and internal motion, $C_I(t)$.

The correlation function $C_I(t)$ for internal motion will not usually decay to zero as this motion is normally restricted and so the internuclear C-H vector cannot take on all possible orientations. This steady state limit, S^2, is known as the (squared) generalised order parameter. If the motion is unrestricted, it will be zero, with deviations from this indicating the degree of restriction.

The spectral density can be written in terms of S^2 and the correlation times τ_M for molecular tumbling and τ_e for internal motion.

$$J(\omega) = \frac{2}{5}\left(\frac{S^2\tau_M}{1+(\tau_M\omega)^2} + \frac{(1-S^2)\tau}{1+(\tau\omega)^2}\right) \tag{3.1}$$

$$\tau^{-1} = \tau_M^{-1} + \tau_e^{-1} \tag{3.2}$$

Experimental results consisting of relaxation data at various frequencies can then be used, in a least squares procedure, to calculate S^2, τ_M and τ_e. In practice this only works for proteins or large molecules where $\tau_e < 0.1\ \tau_M$. If τ_e is of a similar magnitude to τ_M then the fit becomes insensitive to the value of τ_e making it difficult to obtain a valid fit to the data. This is the situation for small molecules where molecular tumbling is fairly rapid. In this case it is best to use an independently determined τ_M and to fix τ_e at zero.[9]

EXPERIMENTAL

^{13}C and ^{1}H nmr assignments of panose at 300 K in D_2O were achieved using standard 2D COSY, TOCSY and HSQC pulse sequences at 400 MHz on a varian Unity400 spectrometer fitted with an inverse detection probe. A series of 15 1D-TOCSY spectra were recorded at 500 MHz with mixing times ranging from 10 ms to 150 ms and using a selective gaussian pulse centered on each of the anomeric protons in turn.

A standard inversion recovery sequence was used to obtain T_1 relaxation times for all the carbons of panose at 200, 300 and 400 MHz. The procedure of Kowalewski and Widmalm[9] in which the value of τ_e is set to zero was used to calculate Lipari and Szabo squared generalized order parameters, S^2. A value of 0.2 ns, obtained from molecular dynamics simulations, was used for τ_M.

RESULTS AND DISCUSSION

While most of the nmr assignments of panose and related compounds are available in the literature, a full ^{13}C and ^{1}H assignment, using modern experimental techniques, is yet to appear. Typically for an oligosaccharide, the proton spectrum of panose has a downfield set of peaks corresponding to the anomeric protons and a poorly resolved set of upfield peaks for the remaining protons. The reducing terminal H-1α signal is very small while H-1β shows strong coupling to H-2. The remaining two anomeric protons are assigned on the knowledge that the $\alpha(1\rightarrow4)$ linkage peaks are always

upfield of those from α(1-6) linkages. Using a series of 1D-TOCSY experiments it was possible to assign unambiguously all the ring protons. Figure 2 shows the assignment of the central residue. Following the assignment of the proton spectrum, the ^{13}C assignment was straightforward. A gradient HSQC (Fig. 3) spectrum allowed all the ^{13}C peaks to be assigned including some which were overlapped. Furthermore the anomeric effect of the reducing sugar was seen in the central residue with the ring carbons being ''twinned''. The full set of ^{1}H and ^{13}C assignments are given in Tables 1 and 2.

^{13}C T1 relaxation times measured at 200, 300 and 400 MHz are given in Table 3, together with the calculated order parameters. Because of signal overlap, it was not possible to measure the relaxation time of C_3 on residue 1 and 3. Inspection of these results shows that, as expected, the relaxation time increases with field strength. We also note that the methylene carbons have much shorter relaxation times.

Of greater interest are the order parameters, which give an indication of the molecular motion of the molecule. All the carbons of the central

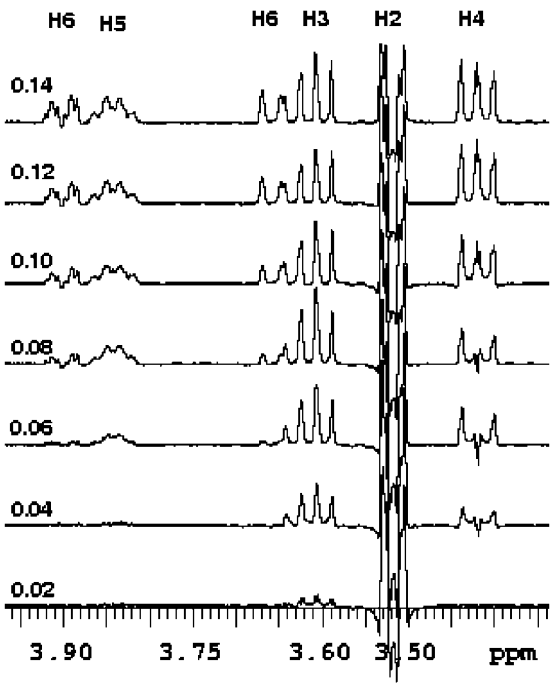

Figure 2. TOCSY 1D spectra of central residue at different mixing times (ms) upon irradiation of anomeric proton at δ 5.36.

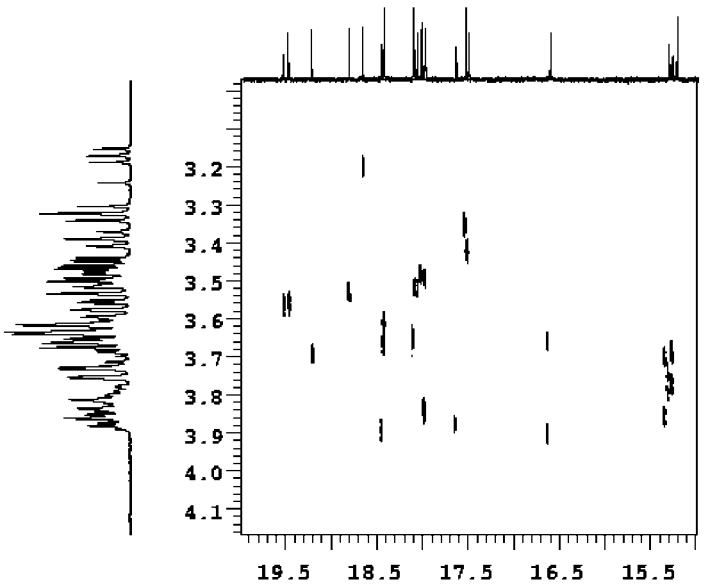

Figure 3. Enlarged portion of a gradient HSQC spectrum showing proton/carbon correlations.

Table 1. ^{1}H Assignments for Panose (Values in *ppm*)

	H1	H2	H3	H4	H5	$H6_1$	$H6_2$
α-Reducing ring	5.19	3.54	3.94	3.61	3.92	3.84	3.78
β-Reducing ring	4.61	3.24	3.74	3.61	3.57	3.74	3.90
Central ring	5.36	3.56	3.65	3.46	3.88	3.94	3.70
Non-reducing ring	4.92	3.53	3.71	3.39	3.68	3.82	3.73

Table 2. ^{13}C Assignments for Panose (Values in *ppm*)

	C1	C2	C3	C4	C5	C6
α-Reducing terminus	93.3	72.7	74.6	78.9	71.4	62.2
β-Reducing terminus	97.2	75.4	77.6	78.6	76.0	62.3
Central ring (β)	101.1	73.1	74.5	70.9	72.8	67.2
Central ring (α)	101.2	73.2	74.5	70.9		
Non-reducing terminus	99.5	72.9	74.5	71.0	73.3	62.0

Some of the central ring carbons exhibited anomeric splitting; these are the alpha peaks.

Table 3. ^{13}C Chemical Shifts, T_1 Relaxation Times, and Order Parameters for Panose

Ring	Carbon	T_1 (200 MHz) (s)	T_1 (300 MHz) (s)	T_1 (400 MHz) (s)	S^2
1	C1	0.40(2)	0.52(3)	0.61(6)	0.49(2)
	C2	0.37(2)	0.54(3)	0.57(6)	0.57(2)
	C3	0.33(1)	0.49(3)	0.55(4)	0.61(2)
	C4	0.32(2)	0.50(2)	0.49(4)	0.57(2)
	C5	0.39(3)	0.45(2)	0.38(3)	0.62(2)
	C6	0.15(1)	0.21(2)	0.21(7)	0.71(2)
	C1(α)	0.35(3)	0.37(1)	0.46(1)	
	C2(α)		0.45(2)	0.56(4)	
	C3(α)	0.31(1)	0.49(3)	0.55(2)	
	C4(α)	0.36(4)	0.52(2)	0.40(1)	
	C5(α)	0.31(3)	0.48(4)	0.51(2)	
	C6(α)	0.15(1)	0.20(2)	0.29(2)	
2	C1	0.28(2)	0.37(2)	0.41(3)	0.74(3)
	C2	0.25(1)	0.38(3)	0.38(3)	0.85(3)
	C3				
	C4	0.24(1)	0.33(2)	0.38(4)	0.93(2)
	C5		0.38(2)	0.36(3)	0.80(1)
	C6	0.15(1)	0.20(1)	0.21(1)	0.92(2)
	C1(α)	0.24(1)	0.34(1)	0.37(2)	
	C2(α)	0.28(2)	0.36(2)	0.34(2)	
3	C1	0.36(2)	0.48(2)	0.50(4)	0.59(2)
	C2	0.27(1)	0.41(1)	0.45(2)	0.72(1)
	C4	0.30(2)	0.41(2)	0.46(3)	0.69(2)
	C5	0.28(1)	0.40(1)	0.46(6)	0.76(2)
	C6	0.20(1)	0.29(2)	0.21(1)	0.54(1)

sugar residue have very similar order parameters indicating that this residue does not exhibit much segmental motion. In addition the order parameters of this residue are significantly higher than the order parameters of the two terminal sugar residues indicating that the motion of this residue is more restricted. An exception to this general observation is C6. As these are methylene carbons they would be expected to have increased freedom of motion. This is also seen for C6 of residue 3. The motion of C6 in residue 2 is more restricted than the equivalent atoms in residue 2 but this is expected as this is the point of attachment of the α(1-6) glycosidic bond. Surprisingly, the motion of C6 in residue 1 is also severely restricted. One explanation for this is hydrogen bonding. The crystal structure[10,11] of panose shows a bifurcated hydrogen bond between O6 of residue 1 and O1

and O2 of residue 3. These hydrogen bonds are also observed, although transiently, in our molecular dynamics simulations. A consequence of these hydrogen bonds is that the orientation of the primary alcohol of the reducing sugar is forced into a high-energy eclipsed rotamer.

ACKNOWLEDGMENTS

Financial support of the University of Cape Town, the National Research Foundation and the United States Department of Agriculture (USDA-ARS grant no. 58–4012–5-F120 to KJN) is gratefully acknowledged.

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Received November 1, 2001
Accepted April 20, 2002