ELSEVIER

Carbohydrate Research 275 (1995) 391–401

CARBOHYDRATE RESEARCH

Note

Conformational analysis of sucrose octasulfate by high resolution nuclear magnetic resonance spectroscopy

Umesh R. Desai, Ioncho R. Vlahov, Azra Pervin, Robert J. Linhardt *

Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, IA 52242, USA

Received 3 May 1994; accepted in revised form 1 April 1995

Keywords: Sucrose octasulfate; Sucralfate; Carafate; Fibroblast growth factor; NMR spectroscopy; Karplus equation; Dihedral angles; Average solution conformation

The aluminum salt of sucrose octasulfate (SOS) is a drug called Sucralfate (Carafate), which is widely used for the treatment of duodenal ulcers. Folkman et al. [1] have recently suggested that Sucralfate binds to fibroblast growth factor (FGF), protecting it from denaturation in the strongly acidic conditions present in the stomach. This hypothesis has generated considerable interest in sucrose octasulfate and its interaction with FGF. The sodium and potassium salts of SOS also bind FGF [2], but these differ from Sucralfate in being water soluble and are more easily studied.

FGF in tissue binds to endogenous glycosaminoglycans. It is often formulated with a highly charged glycosaminoglycan, heparin, to improve its stability [3]. The minimum FGF binding site within heparin is a tetrasaccharide having the same numbers of negative charges as SOS [4]. FGF is considered to be a prototypical heparin binding growth factor, suggesting that the aluminum salt of SOS may be acting as a heparin mimetic. Heparin is a polydisperse, microheterogenous polysaccharidic drug that is considerably more structurally complex than SOS. While single crystal X-ray data are not available for heparin, the high resolution crystal structure of SOS has been determined [5]. More recently, the structure of a SOS-FGF co-crystal has been reported [6].

* Corresponding author.

Elsevier Science Ltd.
SSDI 0008-6215(95)00159-X

SOS and heparin appear to bind the same site within FGF [2,6]. SOS represents an excellent model compound for studying heparin binding to FGF in the absence of crystallographic data on heparin, or the FGF-binding heparin tetrasaccharide [4], or the complex of heparin with FGF. An improved understanding of the solution conformation of SOS will be helpful in studying the interaction of SOS and FGF. This information might also lead to an improved understanding of the interaction of FGF with heparin and its endogenous glycosaminoglycan receptors.

NMR spectroscopy has provided valuable information on both the solution conformation and the conformational flexibility of sucrose. The long held view that sucrose was rigid around its interglycosidic bond has recently been brought into question through the use of NMR spectroscopy [7,8]. Most recently, the solution conformation of sucrose and its conformational mobility have been examined using sophisticated ^{13}C and ^{1}H NMR methods [9,10]. Theoretical steady state NOE data on sucrose do not support a single conformation model but rather conformational averaging [11]. Studies using molecular mechanics and dynamics identified multiple low energy conformations further supporting its flexibility [12,13]. Studies on sucrose, sucrose octaacetate and their *C*-glycosides have compared their preferred solution conformations to their solid state conformations measured from single crystal X-ray analysis [14–16]. In this study, the solution conformation and the X-ray crystal structure of SOS are compared. A preliminary examination of conformational flexibility of SOS is also made.

1. Experimental

NMR spectroscopy of sucrose octasulfate.—Sucrose octasulfate (potassium salt) was purchased from Toronto Research Chemicals Inc. (Toronto, Canada). All NMR experiments were performed using a Bruker AMX 600 (Spectrospin AG, Switzerland) spectrometer operating at 600 MHz for proton and 150 MHz for carbon. Sucrose octasulfate potassium salt (2 mg) was dissolved in distilled, deionized water (500 μL) giving a pH of 7 and filtered through a 0.45 μm membrane. The solution was lyophilized and the remaining traces of water in the resulting solid were exchanged in $^{2}H_2O$ (99.9% atom ^{2}H, Sigma Chemical Co., St. Louis, MO) by repeated lyophilization. After three exchanges, the solid was dissolved in 500 μL of $^{2}H_2O$ (99.96% atom ^{2}H) for NMR experiments. The ^{1}H NMR experiments were conducted at different temperatures with a digital resolution of 0.123 Hz/point. A line broadening factor of 0.24 Hz was used prior to Fourier transformation. The ^{13}C spectra were acquired with broadband proton noise decoupling. The carbon signal of the trimethylsilyl portion of the 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt was set to 0 ppm as reference for ^{13}C NMR spectra. The assignment of ^{1}H and ^{13}C NMR spectra and the conformational analysis of the furanoside moiety of SOS were carried out using two-dimensional experiments such as COSY, NOESY and inverse heteronuclear correlation [17,18]. Mixing times of 300, 850 and 900 ms were used in NOESY experiments. COSY used a 90° pulse (8.8 μs). The pulse programs utilized standard Bruker software. The data for COSY and NOESY experiments were acquired using 1024 data points in the f2 dimension and 256 data points in the f1 dimension and Fourier transformed into a

1K × 1K data matrix using a sine-bell window function. The data for inverse heteronuclear correlation were acquired using 64 data points in the f1 dimension and 1024 data points in the f2 dimension, followed by processing to generate a 1024 × 256 data matrix.

2. Results and discussion

The objective of this study was two-fold: (1) To examine the average solution conformation of SOS (Fig. 1) and to compare this conformation to the solid state conformation determined by X-ray crystallography [5]; and (2) To study the conformational flexibility of SOS and compare SOS flexibility to that recently observed in sucrose [7–10]. NMR spectroscopy is useful for identifying the microenvironment around a nuclear spin, giving valuable information about the time-averaged molecular conformation.

The high resolution 600 MHz ^{1}H NMR spectrum of SOS in ^2H_2O at 298 K, shown in Fig. 2, was assigned and these assignments were confirmed using two-dimensional (2D) NMR experiments including *J*-correlated spectroscopy (COSY) (spectrum not shown). The doublets in the ^{1}H NMR spectrum of SOS (Fig. 2) at δ 5.82 and 5.14 corresponding to the H-1 of glucopyranoside and H-3′ of fructofuranoside rings, respectively, served as starting points in the assignment of ring protons. The ^{1}H chemical shift assignments (Table 1) are consistent with those previously reported for SOS [19].

In an aqueous solution of sucrose all the hydroxyl groups on both the glucopyranoside and fructofuranoside rings are equatorial [7–10]. Similar observations have recently been made for sucrose octaacetate in deuterochloroform [14,15]. The coupling constants (Table 1), obtained at 298 K by first-order analysis of the ^{1}H NMR spectrum of SOS, demonstrate that in solution there is an axial-equatorial relationship between H-1 and H-2, and the *trans*-diaxial relationship between H-3–H-2, H-3–H-4 and H-4–H-5 of the glucopyranoside ring (Fig. 1). Thus, the average solution state conformation of the glucopyranose ring of SOS is consistent with the $^4C_1$ chair form. The same solution and solid state $^4C_1$ conformation is observed for the glucopyranoside residue in sucrose and sucrose octaacetate [7–10,16,20]. The equal and relatively small $^3J_{H5,H6a}$ and $^3J_{H5,H6b}$ values (Table 1) suggest that the C-6 sulfooxymethyl group adopts a solution orientation with H-5 *syn*-clinal to H-6$_a$ and H-6$_b$. Thus, the C-6 sulfate group and H-5 are *anti*-periplanar. In the glucopyranoside portion of SOS, the solution and solid state conformations are identical.

The crystal structure of SOS shows the fructofuranoside ring in the $^{5'}T_{4'}$ twisted form with C-4′ *exo*-oriented to the C-5′–C-6′ bond (Table 2) [5]. Sucrose and sucrose octaacetate show different crystal structures with the fructofuranoside in the $^{4'}T_{3'}$ and $^{3'}_{4'}T$ conformations respectively [14–16,20]. Several structural features of the furanoside ring of SOS act to stabilize the solution conformation of this five-membered ring (Fig. 1). All the substituents are equatorial, eliminating unfavorable steric and charge–charge 1,3-diaxial interactions. The two vicinal sulfate groups, situated in a *trans*-orientation, are *syn*-clinal to one another. Most importantly, only a single unfavorable interaction is

Table 1
Chemical shift assignments and coupling constants (in Hz) at various temperatures for sucrose octasulfate

Temperature (K)	Chemical shifts (δ) and J (Hz) [a]													
	H-1 ($^3J_{H1,H2}$)	H-2 ($^3J_{H2,H3}$)	H-3 ($^3J_{H3,H4}$)	H-4 ($^3J_{H4,H5}$)	H-5 ($^3J_{H5,H6a}$)	H-6a ($^2J_{H6a,H6b}$)	H-6b ($^3J_{H5,H6b}$)	H-1′a ($^2J_{H1'a,H1'b}$)	H-1′b	H-3′ ($^3J_{H3',H4'}$)	H-4′ ($^3J_{H4',H5'}$)	H-5′ ($^3J_{H5',H6'a}$)	H-6′a ($^2J_{H6'a,H6'b}$)	H-6′b ($^3J_{H6'b,H5'}$)
278	5.83	4.42	4.74	4.47	4.38	4.40	4.42	4.24	4.33	5.14	4.79	4.45	4.32	4.61
	d	dd	dd	dd	ddd	ddd	ddd	d	d	d	dd	ddd	dd	dd
	(3.5)	(9.6)	(9.0)	(9.0)	(2.8)	(10)	(2.8)	(10.8)	(10.8)	(7.8)	(7.8)	(9.0)	(11.4)	(3.6)
288	5.82	4.42	4.73	4.49	4.36	4.39	4.42	4.25	4.33	5.13	4.77	4.45	4.31	4.61
	d	dd	dd	dd	ddd	ddd	ddd	d	d	d	dd	ddd	dd	dd
	(3.5)	(10)	(9.0)	(9.0)	(2.8)	(10)	(2.8)	(10.8)	(10.8)	(7.8)	(7.8)	(9.0)	(11.4)	(2.4)
298	5.81	4.41	4.74	4.48	4.38	5.39	4.41	4.25	4.33	5.14	4.77	4.46	4.31	4.61
	d	dd	dd	dd	ddd	ddd	ddd	d	d	d	dd	ddd	dd	dd
	(3.5)	(10)	(9.0)	(9.0)	(2.8)	(9.9)	(2.8)	(10.7)	(10.7)	(7.8)	(7.8)	(9.0)	(11.6)	(2.4)
308	5.81	4.41	4.73	4.48	4.35	4.39	4.41	4.24	4.31	5.13	4.77	4.45	4.30	4.61
	d	dd	dd	dd	dd	ddd	ddd	d	d	d	dd	ddd	dd	dd
	(3.5)	(10)	(9.0)	(9.0)	(2.8)	(9.9)	(2.8)	(10.2)	(10.2)	(7.8)	(7.8)	(9.0)	(11.4)	(2.4)
318	5.82	4.41	4.74	4.48	4.36	4.39	4.41	4.24	4.33	5.13	4.76	4.46	4.31	4.61
	d	dd	dd	dd	ddd	ddd	ddd	d	d	d	dd	ddd	dd	dd
	(3.5)	(9.8)	(9.0)	(9.0)	(2.8)	(9.8)	(2.8)	(10.8)	(10.8)	(7.8)	(7.8)	(9.0)	(11.4)	(2.4)

[a] The accuracy of the coupling constants is defined by the digital resolution of 0.123 Hz/pt.

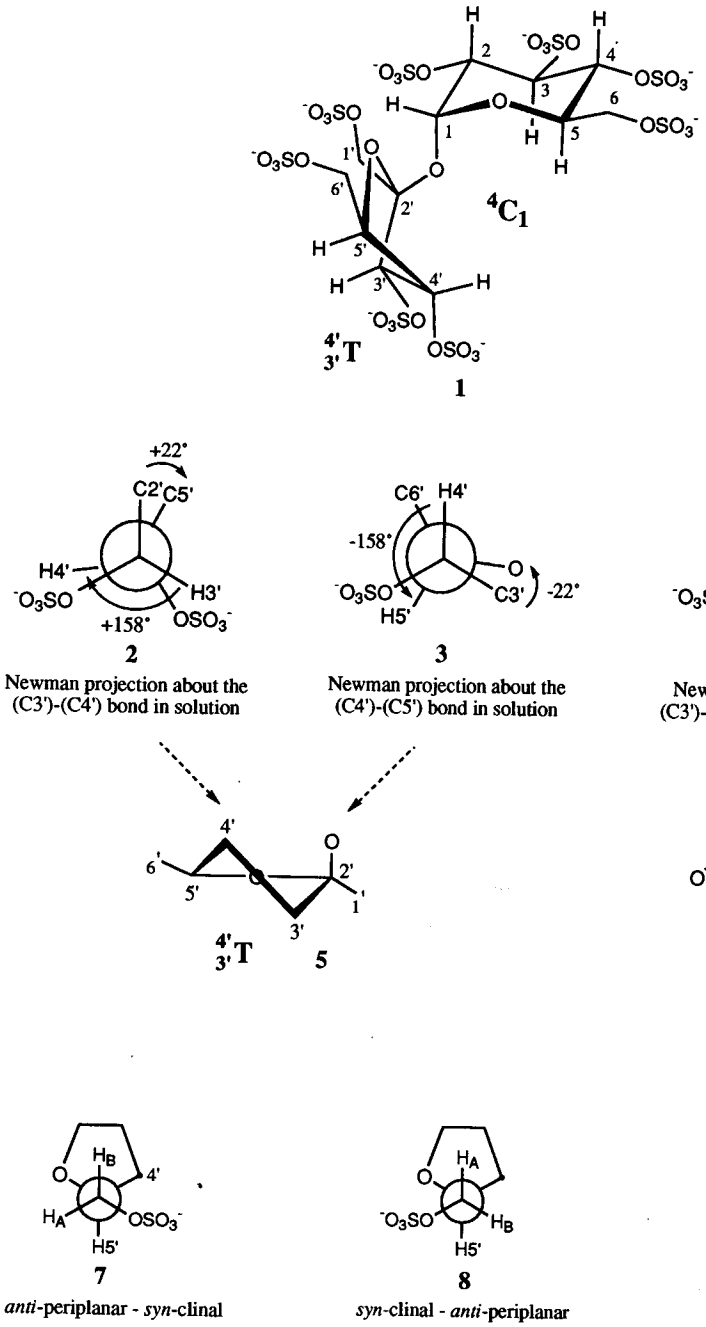

Fig. 1. Solid-state and solution conformations of sucrose octasulfate. The average solution conformation of SOS (**1**) determined by ^{1}H NMR. The glucopyranoside ring is in the $^4C_1$ chair form and the fructopyranoside ring in the $^{4'}_{3'}T$ twisted conformation. Newman projections of portions of the fructofuranoside ring of sucrose octasulfate are shown in **2** and **3** in solution in ^2H_2O and **4** in the solid state. Projection **2** is looking down the C-3′–C-4′ bond, projection **3** is looking down the C-4′–C-5′ bond and projection **4** is looking down the C-3′–C-4′ bond. The average conformation of the furanoside ring is shown in the $^{4'}_{3'}T$ conformation as it appears in solution in ^2H_2O (**5**). Its conformation in the single crystal X-ray structure is shown in **6**. Newman projections **7**, **8** and **9** show the three possible staggered rotamers about the C-6–C-5′ bond.

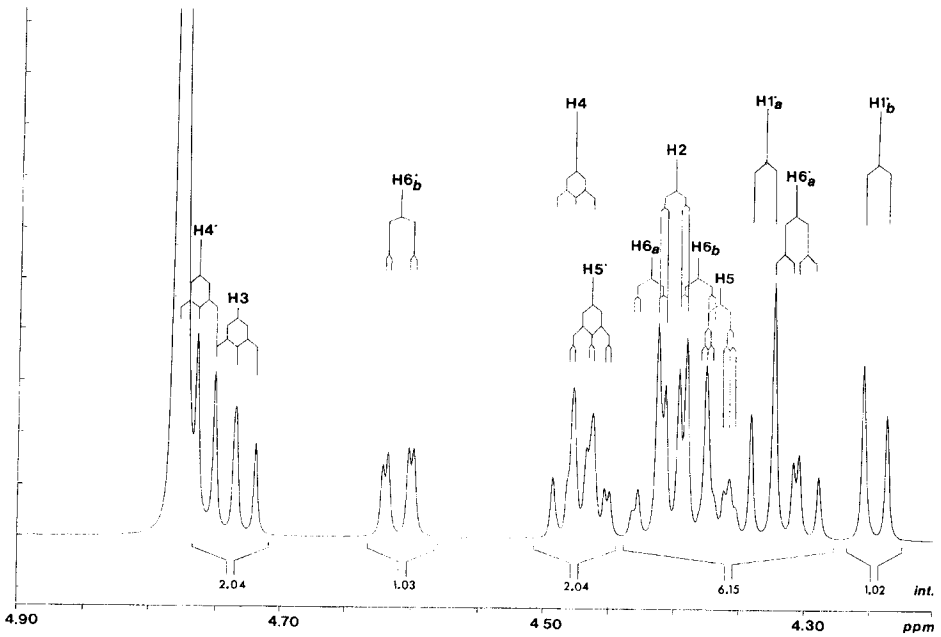

Fig. 2. The ^{1}H NMR spectrum (600 MHz) of SOS (2 mg/mL) in $^{2}H_2O$ at 298 K. The $^{2}H_2O$ signal at 4.8 ppm is off-scale.

observed, corresponding to the *syn*-periplanar orientation between the anomeric oxygen and the sulfate at C-3′. An ${}_{3'}E$ envelope or any twisted (T) conformation in which C-3′ is *exo* should serve to decrease this unfavorable interaction. In the ${}_{3'}E$ conformer, $^{3}J_{H3',H4'}$ should be different from $^{3}J_{H4',H5'}$. The ^{1}H NMR of SOS rules out this envelope conformation since $^{3}J_{H3',H4'}$ and $^{3}J_{H4',H5'}$ are identical (Table 1). An alternate conformation leading to a decrease in the unfavorable C-3′-sulfate–anomeric oxygen interaction would place C-3′ *endo* in either ${}^{3'}E$ or T conformers. These conformations would result in very small values for $^{3}J_{H3',H4'}$ and $^{3}J_{H4',H5'}$. This is not in agreement with the observed J values being greater than 6 Hz (Table 1). Thus, a twisted conformer with an *exo* C-3′ must be responsible for decreasing the unfavorable interaction between the C-3′-sulfate

Table 2
Fructofuranoside conformations in sucrose derivatives

	SOS [a]	Sucrose octaacetate [b]	Sucrose [c]
Solution	${}^{4'}_{3'}T$	${}^{4'}_{3'}T$	${}^{4'}_{5'}T$
	$^{3}J_{H1',H4'} = {}^{3}J_{H4',H5'}$ = 7.8 Hz	$^{3}J_{H3',H4'} = {}^{3}J_{H4',H5'}$ = 6.4 Hz	$^{3}J_{H3',H4'} = 8$ Hz, $^{3}J_{H4',H5'}$ = 8.6 Hz
Solid state	${}^{5'}T_{4'}$	${}^{3'}_{4'}T$	${}^{4'}T_{3'}$

[a] Solution structure by ^{1}H NMR in $^{2}H_2O$ and solid-state structure from single crystal X-ray [5].
[b] Solution structure by ^{1}H NMR in $C^{2}HCl_3$ and solid-state structure from single crystal X-ray [20].
[c] Solution structure by ^{1}H NMR in $^{2}H_2O$ [15,22] and solid-state structure from single crystal X-ray [16].

group and the anomeric oxygen. These same arguments also apply to sucrose octaacetate, recently examined in our laboratory by ^{1}H NMR spectroscopy, suggesting its furanoside ring also occupies a twisted conformation (Table 2). The $^3J_{H3',H4'}$ and $^3J_{H4',H5'}$ values are identical and large (7.8 Hz) (Table 1). Assuming that the ring oxygen, C-2′ and C-5′ are situated in the same plane, one can conclude that the conformation of the fructofuranoside ring of SOS in ^2H_2O is ${}^{4'}_{3'}T$ (Table 2). This suggests that the solution conformation (Fig. 1, **5**) of the fructofuranose residue is different from its solid state conformation (Fig. 1, **6**). The torsion angle H-3′–C-3′–C-4′–H-4′ determined from the Karplus equation [21] using $^3J_{H3',H4'}$ of 7.8 Hz (Table 1) is +158°. This simple, early version of the Karplus equation gives sufficient accuracy in calculating angles for an average conformation. A more complex calculation [22] would lead to the same conclusion regarding the average conformation of SOS. From the Newman projection viewed along the C-3′–C-4′ bond, the dihedral angle C-2′–C-3′–C-4′–C-5′ is +22° (Fig. 1, **2**). This same dihedral angle is −22° in the X-ray crystal structure (Fig. 1, **4**). Similarly, the dihedral angle H-4′–C-4′–C-5′–H-5′ determined from the Karplus equation using $^3J_{H4',H5'}$ of 7.8 Hz is −158° (Table 1). From the Newman projection viewed along the C-4–C-5′ bond, the dihedral angle C-3′–C-4′–C-5′–O-5′ is −22° (Fig. 1, **3**). In the crystal structure this same dihedral angle is +30°. The same *absolute* value for the C-2′–C-3′–C-4′–C-5′ and the C-3′–C-4′–C-5′–O-5 dihedral angles, of 22°, as expected due to conformational averaging in solution NMR, demonstrating the C_2 point group symmetry of the furanoside ring in its ${}^{4'}_{3'}T$ conformation. The dihedral angles taken from the X-ray structure of SOS do not show C_2 point group symmetry for the furanoside ring in the ${}^{5'}T_{4'}$ conformation. The fructofuranoside residue in sucrose adopts ${}^{4'}_{5'}T$ conformation in solution probably due to the presence of intramolecular hydrogen bonding [23–25]. The C-2-oxygen acts as an acceptor for either 1′-OH or 3′-OH. The switch from one form to the other requires a minor rotation around the interglycosidic linkage and takes place with conservation of the overall molecular geometry. The ${}^{4'}_{5'}T$ conformation assumes a shorter distance between the C2-oxygen and 3′-OH when compared to the ${}^{4'}_{3'}T$ conformer.

Table 3
Chemical shift assignments of the carbon signals of sucrose octasulfate

	Chemical shifts (ppm) at various temperatures						
Atom	278 K	288 K	298 K	308 K	318 K	328 K	338 K
C-1	92.61	92.61	92.61	92.62	92.64	92.66	92.68
C-2	77.07	77.07	77.07	77.08	77.09	77.10	77.11
C-3	78.59	78.58	78.57	78.57	78.57	78.58	78.58
C-4	76.61	76.66	76.72	76.78	76.85	76.91	76.97
C-5	72.15	72.16	72.19	72.22	72.27	72.33	72.40
C-6	69.12	69.42	69.13	69.14	69.16	69.19	69.22
C-1′	69.65	69.65	69.67	69.69	69.72	69.76	69.80
C-2′	104.77	104.77	104.78	104.80	104.83	104.87	104.93
C-3′	81.14	81.21	81.28	81.34	81.43	81.51	81.61
C-4′	80.86	80.96	81.06	81.15	81.25	81.35	81.44
C-5′	81.14	81.18	81.22	81.27	81.32	81.37	81.44
C-6′	71.71	71.78	71.83	71.87	71.90	71.93	71.96

The ^{1}H NMR chemical shifts are sensitive to their microenvironment. If a molecule is flexible, a change in temperature will often result in a change in the equilibrium between conformers, changing the microenvironment of the protons present and affording a change in their chemical shifts. ^{1}H NMR spectra were recorded at temperatures ranging

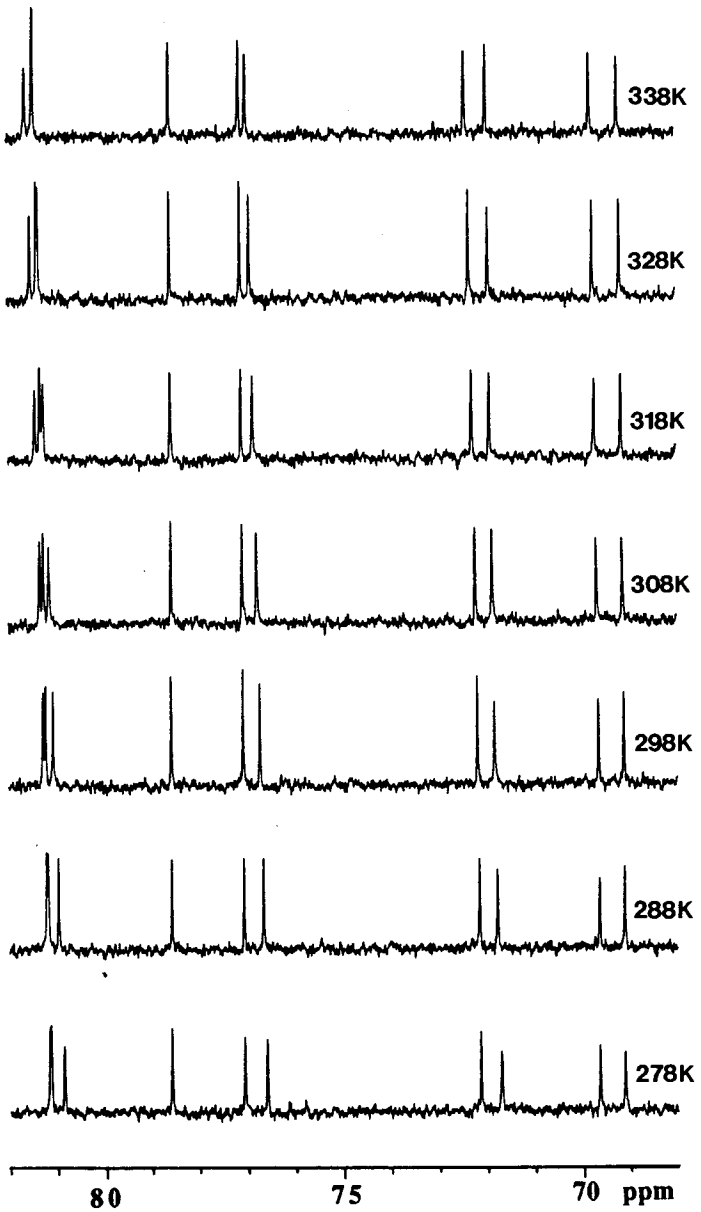

Fig. 3. The ^{13}C NMR spectra (150 MHz) of SOS in the temperature range 278–338 K.

from 278–318 K (Table 1). The H-1, H-3, and H-4 signals of the glucopyranoside ring and all protons of the fructofuranoside ring have distinct positions and hence, their chemical shifts are easy to monitor. Glucopyranoside ring protons become slightly shielded with increased temperature. The H-3 signal is slightly deshielded, but no major changes in chemical shifts or coupling constants of the glucopyranoside ring protons are observed. The fructofuranoside ring protons also become slightly shielded with increasing temperature. The coupling constants for the fructofuranoside residue remain virtually unchanged with increasing temperature. No evidence has been found from these data indicating the flexibility of SOS, possibly because the temperature range examined was too narrow or the conformers present were energetically equivalent. The sulfate groups certainly decrease the conformational flexibility of SOS because of the repulsive electrostatic interaction between the negative charges.

The ^{13}C resonance assignments are presented in Table 3. The ^{13}C NMR spectrum of SOS at 298 K was assigned using inverse heteronuclear correlation spectroscopy (spectrum not shown). To study SOS flexibility, ^{13}C NMR spectra of SOS were recorded at temperatures ranging from 278 to 338 K to provide information about ring fluctuations (Fig. 3). The C-1, C-2, C-3 and C-6 signals of the glucopyranoside ring are almost invariant, while the C-4 and C-5 signals show a slight deshielding with increasing temperature. Similar analysis of the shifts of the fructofuranoside carbons shows that C-4′ and C-3′ are the most temperature sensitive followed by the C-5′, C-6′, and C-2′ signals (Fig. 3). The C-4′ and C-3′ signals are deshielded with increasing temperature, suggesting that there is some conformational fluctuation at these atoms.

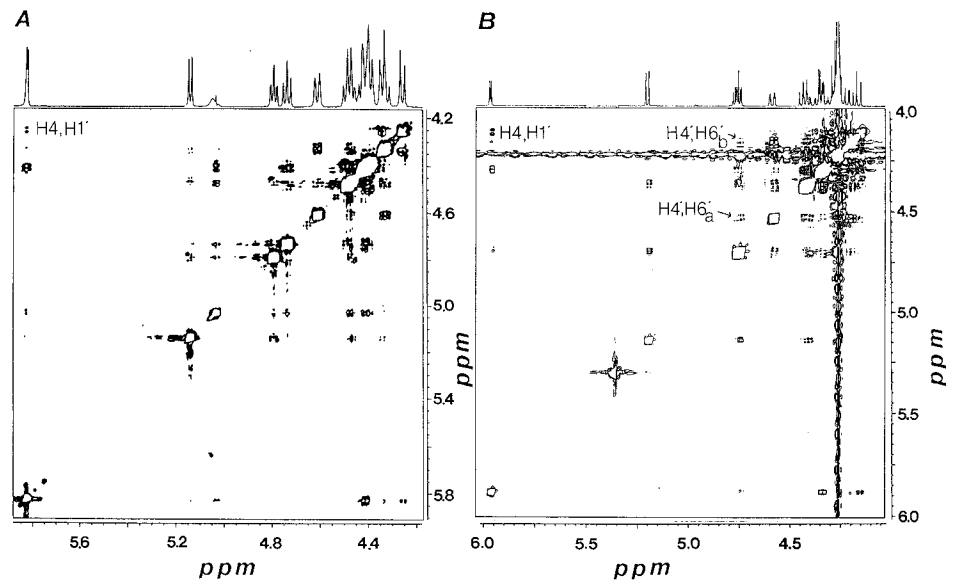

Fig. 4. NOESY spectra of SOS in $^{2}H_2O$ at 278 K (**A**) and at 338 K (**B**). The experiments were performed with a 350 ms mixing time and 4 s recovery delay. The spectra were processed using a sine-bell window function and symmetrized. The dipolar connectivity (fructofuranose-H1′, glucopyranose-H1) is maintained at both temperatures suggesting limited conformational mobility around the interglycosidic bond.

The space available for torsion changes around the interglycosidic linkage is very limited based on repulsive electrostatic considerations, and hence, it is reasonable to propose similar conformations in the range 278–338 K. The X-ray structure of SOS suggests closeness in space of the H-1 proton of the glucopyranoside ring and both of the H-1′ protons of the fructofuranoside ring. Hence, nuclear Overhauser enhancement (NOE) spectroscopy was used to examine the flexibility of the interglycosidic bond. NOESY was performed under otherwise identical conditions at 278 K and 338 K (Fig. 4). In both spectra, a cross-peak due to the H-1 and one of the H-1′ signals was observed, indicating dipolar connectivity at both 278 and 338 K. Thus, the average position of these two protons remain spatially close in the temperature range studied, suggesting that flexibility around the interglycosidic linkage is considerably less than flexibility further away from this linkage. The three staggered conformers **7**, **8** and **9** (Fig. 1) can be distinguished by examining the NOE cross-peaks corresponding to protons in the fructofuranoside ring (Fig. 3B). The H-4′ to H-6′$_a$ and H-4′ to H-6′$_b$ enhancements are approximately equivalent, unambiguously corresponding to the *syn*-clinal-*anti*-periplanar relationship of rotamer **8**. The larger $^3J_{H5',H6'b}$ and the smaller $^3J_{H5',H6'a}$ values provide additional evidence for the preference of rotamer **8** in solution; in the crystal structure rotamer **7** was observed. Rotamer **8** is also the preferred orientation of the 6′-hydroxymethyl group in sucrose.

In conclusion, these studies demonstrate that the average solution conformation of the furanoside ring in SOS is different from that in the solid state determined by X-ray crystallography. These are similar to the differences that have been observed between the solution and solid-state conformations of sucrose [15,20] and sucrose octaacetate [14–16]. This study demonstrates that conventional NMR analysis is a simple yet elegant means for establishing the ring conformations of SOS in solution.

Acknowledgements

The authors thank Dr Ron Hileman, Dr Toshihiko Toida and Mr David Larson for their technical assistance. This work was supported by Wyeth Ayerst and the NIH (GM38060).

References

[1] J. Folkman, S. Szabo, M. Stovroff, P. McNeil, W. Li, and Y. Shing, *Ann. Surg.*, 214 (1991) 414–425.
[2] D.B. Volkin, A.M. Verticelli, K.E. Marfia, C.J. Burke, H. Mach, and C.R. Middaugh, *Biochim. Biophys. Acta*, 1203 (1993) 18–26.
[3] D.B. Volkin, P.K. Tsai, J.M. Dabora, J.O. Gress, C.J. Burke, R.J. Linhardt, and C.R. Middaugh, *Arch. Biochem. Biophys.*, 300 (1993) 30–41.
[4] H. Mach, D.B. Volkin, C.J. Burke, C.R. Middaugh, R.J. Linhardt, J. Fromm, D. Loganathan, and L. Mattsson, *Biochemistry*, 32 (1993) 5480–5489.
[5] Y. Nawata, K. Ochi, M. Shiba, and K. Morita, *Acta Crystallogr.*, B37 (1981) 246–249.
[6] X. Zhu, B.T. Hsu, and D. Rees, *Structure*, 1 (1993) 27–34.

[7] L. Poppel and H. van Halbeek, *J. Am. Chem. Soc.*, 114 (1992) 1092–1094.
[8] B. Adams and L. Lerner, *J. Am. Chem. Soc.*, 114 (1992) 4827–4829.
[9] J.H. Duker and A.S. Serianni, *Carbohydr. Res.*, 249 (1993) 281–303.
[10] D. Grilich and H.-D. Lüdemann, *Z. Naturforsch.*, 48 (1993) 407–413.
[11] C.H. du Penhoat, A. Imberty, N. Roques, V. Michan, J. Mentech, G. Descotes, and S. Pèrez, *J. Am. Chem. Soc.*, 113 (1991) 3720–3727.
[12] V. Tran and J.W. Brady, *Biopolymers*, 29 (1990) 961–976.
[13] V. Tran and J.W. Brady, *Biopolymers*, 29 (1990) 977–997.
[14] D.J. O'Leary and Y. Kishi, *J. Org. Chem.* 58 (1993) 304–306.
[15] D.J. O'Leary and Y. Kishi, *J. Org. Chem.*, 59 (1994) 6629–6636.
[16] J.D. Oliver and L.C. Strickland, *Acta Crystallogr.* C40 (1984) 820–824.
[17] W.P. Aue, E. Bartholdi, and R.R. Ernst, *J. Chem. Phys.*, 64 (1976) 2229–2246.
[18] S. Macura and R.R. Ernst, *Molec. Physics*, 41 (1980) 95–117.
[19] G.L. Silvey, *J. Pharm. Sci.*, 81 (1992) 471–474.
[20] G.M. Brown and H.A. Levy, *Acta Crystallogr.*, B29 (1973) 790–797.
[21] M. Karplus, *J. Am. Chem. Soc.*, 85 (1963) PPO 2870.
[22] C.A.G. Haasnoot, F.A.A.M. De Leeuw, and C. Altona, *Tetrahedron*, 36 (1980) 2783–2792.
[23] J.C. Christofides and D.B. Davis, *J. Chem. Soc. Chem. Commun.*, (1985) 1533–1534.
[24] D.B. Davies and J.C. Christofides, *Carbohyr. Res.*, 163 (1987) 269–274.
[25] E.S. Stevens and C.A. Duda, *J. Am. Chem. Soc.*, 113 (1991) 8622–8627.