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THE SOLUTION CONFORMATION OF SUCROSE: CONCENTRATION AND TEMPERATURE DEPENDENCE

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ABSTRACT

^{13}C-N.m.r. spin–lattice relaxation measurements were used to study molecular motion in aqueous sucrose. Results show that sucrose tumbles anisotropically in solution, and that its conformation is independent of temperature and concentration. Internal rotation occurs with distinctly different rates and activation energies in the three hydroxymethyl groups. Our data are consistent with results of calculations by Bock and Lemieux[6] who predicted that the conformation of aqueous sucrose is similar to the crystal conformation but with the loss of one intramolecular hydrogen-bond.

INTRODUCTION

The crystal structure of sucrose is well known from X-ray[1] and neutron-diffraction[2] studies; however, its solution conformation is controversial. Some published data suggest that sucrose exhibits different conformations in dilute solution and in the crystal, but other evidence favors similar conformations in both environments.

Mathlouthi *et al.*[3–5] interpreted X-ray and Raman data to show that the conformation of aqueous sucrose is concentration-dependent. At low concentrations (below ~0.7M) they found no evidence for intramolecular hydrogen bonds (H-bonds); instead, they suggested that the glucose and fructose rings are bent apart so that sucrose forms H-bonds only to water. As concentration increases, their evidence suggested that sucrose begins to form intramolecular H-bonds; this is accompanied by some twisting about the glycosidic linkage to bring the glucose and fructose rings together. Finally, in the limit of saturated solutions and in amorphous solid sucrose, Mathlouthi *et al.* found that the pattern of H-bonds resembles those in the crystal structure.

Bock and Lemieux[6] used hard-sphere calculations to predict the solution conformation. According to their theoretical results, dilute aqueous sucrose occupies

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a deep, narrow energy-well with one intramolecular H-bond and a conformation close to that in the crystal structure. These predictions were supported by experimental chemical shift and relaxation data[6] from ^{1}H- and ^{13}C-n.m.r. The n.m.r. data provided evidence of conformational rigidity and a single intramolecular H-bond in dilute aqueous sucrose.

We have obtained n.m.r. relaxation data from aqueous sucrose at many different concentrations and temperatures. Our original purpose for obtaining the large data-set was to study correlation times and spectral-density functions; an analysis in terms of these parameters will be published elsewhere. In this paper we address only the problem of solution conformation. We have used essentially the same analysis as Bock and Lemieux[6], and we are in agreement with their major conclusions. However, our data are more accurate than theirs, and we have studied a wider range of temperatures and concentrations. These improvements allow us to extend their conclusions.

EXPERIMENTAL

Samples. — Solutions were prepared from weighed amounts of reagent-grade sucrose and D_2O (Bio-Rad, 99.8% D). Samples at four sucrose concentrations (1.0, 0.50, 0.25, and 0.10M) were placed in glass n.m.r. sample-tubes, deoxygenated by N_2 bubbling, and then sealed. Relaxation times from sealed solutions stored at 4° showed no measurable change during one year of repeated use.

Instrumentation. — Four n.m.r. spectrometers were used for this work. They were Nicolet models NT-150, NT-200, NT-360, and NT-470 operating at ^{13}C frequencies of 37.74, 50.31, 90.54, and 118.21 MHz with proton decoupling at 150, 200, 360, and 470 MHz, respectively. Probes accepting 12-mm sample-tubes were used in the NT-150 and NT-200, while samples for the NT-360 and NT-470 were 8 mm in diameter. All spectrometers were equipped for computer-controlled, variable sample temperatures.

Before each run, samples were allowed to reach equilibrium at a preset temperature (calibrated within ±2°) in the n.m.r. probe. The proton-decoupler power was set to a level just sufficient to collapse the C-H coupling with a minimum of r.f. heating. The n.m.r. transmitter-frequency was set near the center of the sucrose spectrum (~76 p.p.m. downfield from Me_4Si) and the 180° pulse-width was redetermined for each sample or temperature change. We used 8K data points and a spectral window with a width of 35–40 p.p.m.

Measurements. — Spin–lattice relaxation times (T_1) were measured by using the inversion–recovery pulse-sequence[7] with recycle times approximately equal to $5T_1$. A set of 10–32 (usually 14) different variable-delay times ranging from $<0.1T_1$ to $\sim 5T_1$ were used for each run. The time required to obtain each data-set varied from 5–10 min on M samples to 30 min for 0.10M. Free-induction decays were apodized by using an exponential line-broadening parameter approximately equal to the line width, which varied with temperature and concentration.

After Fourier transformation, T_1 was determined from the data set by a relaxation equation with three adjustable parameters[7], including one that corrects for errors caused by inhomogeneous r.f. fields. All of the data fit the relaxation equation quite well; we find no evidence of non-exponential decay.

Eleven different T_1 values were measured from each experimental run, corresponding to the relaxation times from eleven of the twelve carbon atoms in sucrose. The signal at lowest field in the sucrose spectrum is produced by a quaternary carbon; its T_1 value was not measured because it is much longer than the relaxation times of the other carbon atoms. Accurate measurement of a long T_1 value would require a separate set of experiments with different pulse-delay intervals. For the ring-carbon atoms in M solutions. T_1 ranged from ~0.1 s at 2° to ~0.9 s at 80°. Relaxation times in 0.10M solutions were about twice as long.

The values were measured as a function of temperature (from 2 to 80°), magnetic field (on four different n.m.r. spectrometers) and concentration (0.10, 0.25, 0.50, and 1.0M sucrose in D_2O). Over 1700 individual T_1 measurements were made. So many data-points were recorded that complete results cannot be tabulated conveniently. Therefore, we present our results as tables of averaged data.

RESULTS

The ^{13}C-n.m.r. spectrum of sucrose has been assigned[6]. For convenience we refer to the spectral peaks by number. Each peak is numbered sequentially, beginning at the low-field end of the spectrum. Peak 1, the signal at lowest field, is derived from a quaternary carbon atom. Peaks 2–9 represent –CH(OH)– ring carbon atoms, and peaks 10–12 are from the terminal $-CH_2OH$ groups.

Anisotropic and internal motion may be studied in aqueous sucrose by comparing the spin–lattice relaxation-times of different carbon atoms. To do this successfully, we need greater accuracy than is commonly achieved in individual relaxation-time measurements. Even using extreme care, it is difficult in a single experiment to measure T_1 with a precision better than ±5%. Therefore, the accuracy that we require (~±2%) must be obtained by averaging data from a number of experiments. However, it is inappropriate simply to average the raw data; most of the relaxation times are not directly comparable because they were recorded at different temperatures, concentrations, and fields.

We have chosen to reduce our data to a common basis before combining them. The average relaxation-rate, $1/T_1$, from peaks 2–9 provides an appropriate reference for this purpose because the ring-carbon atoms, taken as a group, constitute a platform about which the various anisotropic and internal motions can be described. Accordingly, we present our data in terms of normalized relaxation-rates $\langle 1/T_1 \rangle$, where $\langle 1/T_1 \rangle$ is defined as the measured relaxation-rate obtained from an individual peak divided by the average $1/T_1$ value of peaks 2–9 as measured during the same run.

If certain limiting conditions apply, normalized relaxation-rates may be averaged, even though they were obtained under a variety of experimental conditions. For example, if $1/T_1$ is directly proportional to solution viscosity and independent of magnetic field-strength, then we should measure the same $\langle 1/T_1 \rangle$ values in each of our experiments. The viscosity condition ensures that the individual relaxation-rates maintain constant relative proportions, so that their temperature and concentration dependence cancels out of the normalized data. To test the assumption that $\langle 1/T_1 \rangle$ is independent of experimental conditions, we compared the averaged, normalized relaxation-rates from various subsets of the entire data-field, namely, from data obtained on each spectrometer, from each sucrose concentration, and from different temperature ranges.

Table I presents the results as a function of temperature and compares them with literature data[8]. Our precision limits (±2% for ring-carbon atoms and ±3% for hydroxymethyl groups) represent two standard deviations of the mean (s.d.m.). One s.d.m. is defined as the standard deviation of the individual $\langle 1/T_1 \rangle$ measurements divided by the square root of the total number of measurements. At the 95% confidence limit, the reported value and the true value differ by less than two s.d.m. if there are no systematic errors.

TABLE I

NORMALIZED ^{13}C-N.M.R. RELAXATION-RATES, $\langle 1/T_1 \rangle$, FROM FOUR TEMPERATURE RANGES COMPARED WITH LITERATURE DATA[a]

Peak[b]	*Assignment*[c]	*Temperature range (this work)*[d]				*A & D*[e]
		2–20°	*20–40°*	*40–60°*	*60–80°*	*55°*
2	1^g	1.03	1.04	1.02	1.04	1.01
3	5^f	1.06	1.07	1.08	1.07	1.06
4	3^f	1.01	1.03	1.04	1.04	1.04
5	4^f	1.00	1.00	0.98	0.97	1.01
6	3^g	0.96	0.95	0.97	0.96	0.97
7	5^g	0.98	0.97	0.98	0.98	0.97
8	2^g	0.99	0.99	0.99	0.98	0.99
9	4^g	0.97	0.97	0.96	0.96	0.96
10	6^f	1.51	1.46	1.39	1.31	1.38
11	1^f	1.72	1.72	1.69	1.66	1.64
12	6^g	1.71	1.66	1.58	1.52	1.54

[a]A normalized relaxation-rate, $\langle 1/T_1 \rangle$ is equal to the measured relaxation-rate, $1/T_1$, for an individual spectral peak, divided by the average relaxation-rate of peaks 2–9, as measured in the same experiment. [b]For covenient reference, peaks are numbered consecutively, beginning at the low-field end of the spectrum. Relaxation rates at peak 1 (carbon 2^f) were not measured. [c]Assignments follow the standard numbering system for sucrose (from ref. 6). [d]Entries are the averages of $\langle 1/T_1 \rangle$ values measured in the indicated temperature ranges. The data-set consisted of 159 different experiments, approximately evenly divided among the four temperature ranges. Error limits (two standard deviations of the mean) are ±2% for peaks 2–9 and ±3% for peaks 10–12. [e]This column presents data originally published by Allerhand and Dohrenwend[8] as absolute relaxation-rates, $1/T_1$. The measurements were made at 55° on a spectrometer with a ^{1}H-n.m.r. frequency of 200 MHz. To facilitate comparison with our results, we have recalculated the data in terms of normalized rates, $\langle 1/T_1 \rangle$.

Comparing one carbon atom with another, Table I shows many significant $\langle 1/T_1 \rangle$ differences. For example, the $\langle 1/T_1 \rangle$ values of peaks 3 and 6 differ by 11%. However, with the exception of peaks 10–12, no significant temperature dependence is evident in the data from individual carbon atoms.

Table II presents the data as a function of concentration. No significant concentration-dependence can be found in data from any of the carbon atoms. Since peaks 2–9 showed no temperature dependence, we averaged all of the data (2 to 80°) for these peaks. Peaks 10–12 are temperature-dependent, but most of the variation occurs at high temperatures. Therefore, to decrease the error limits for peaks 10–12, we used only data from the range 2 and 40°.

We also found a small field-dependence in data from peaks 10–12, but none (within the limits of error) for peaks 2–9. The field dependence was anticipated on theoretical grounds. In effect, we have suppressed the field dependence in Tables I and II by averaging $\langle 1/T_1 \rangle$ values from all four spectrometers. Entries in the tables represent weighted averages approximately corresponding to relaxation rates that would be measured at an intermediate field (that is, with a spectrometer having a ^1H frequency of ~300 MHz). Field dependence contributes to the larger error-limits quoted for peaks 10–12.

Systematic errors are difficult to estimate in relaxation measurements because there are no T_1 calibration standards. We consider that a major advantage of our normalization treatment is its tendency to cancel systematic errors that affect all peaks equally. As a consequence of normalization, any systematic errors that remain in Tables I and II must be those that affect one peak relative to another. One

TABLE II

NORMALIZED RELAXATION-RATES, $\langle 1/T_1 \rangle$, FROM SUCROSE AT FOUR DIFFERENT CONCENTRATIONS IN D_2O[a]

Peak[b]	*Sucrose concentration* (M)			
	1.0	*0.50*	*0.25*	*0.10*
2	1.03	1.03	1.03	1.03
3	1.05	1.08	1.08	1.07
4	1.02	1.03	1.02	1.03
5	0.99	1.00	1.00	0.99
6	0.96	0.96	0.95	0.97
7	0.98	0.97	0.97	0.99
8	0.99	0.99	0.99	0.99
9	0.97	0.96	0.97	0.95
10	1.50	1.47	1.48	1.48
11	1.72	1.69	1.73	1.75
12	1.69	1.67	1.68	1.69

[a]Entries are averages of data from 159 different experiments, approximately evenly divided among the four concentrations. Data from the temperature range 2–80° were used for peaks 2–9. To minimize scatter caused by temperature effects, only data from the temperature range 2–40° were used for peaks 10–12. Error limits (two standard deviations of the mean) are ±2% for peaks 2–9 and ±3% for peaks 10–12. [b]Numbered as in Table I.

possible source of error, unequal pulse excitation at different peak positions, has been largely removed by the three-parameter equation used to fit the inversion–recovery data[7]. Errors could also be caused by poor peak resolution, but this would be a problem only for peaks 6 and 7, which are separated by 8 Hz at the lowest field used. At most temperatures and concentrations, peaks 6 and 7 were reasonably well resolved. However, peak overlap may be expected to narrow slightly the difference between $\langle 1/T_1 \rangle$ values of peaks 6 and 7 without affecting the data from any other peaks.

Allerhand and Dohrenwend[8] recently considered the problem of systematic errors in relaxation measurements with sucrose. They used two entirely different methods to measure relaxation rates; comparing the two, they concluded that systematic errors were unimportant in their data. We can also use their data to test for systematic errors in our measurements, but first the data must be normalized. We averaged both sets of relaxation rates measured by Allerhand and Dohrenwend and then normalized their average rate in the same way as we treated our own data. Table I shows the results. The average deviation between our data and that of Allerhand and Dohrenwend is less than the quoted error limits. Considering the excellent agreement, we feel that systematic errors are unlikely to be significant in our data.

DISCUSSION

In the limiting case of rigid, spherical molecules, dipole–dipole relaxation rates are described by a simple equation. However, if one admits the possibility of anisotropic and internal rotation, the theoretical expressions become extremely complex[9–11]. It is inconvenient to reproduce here the full set of theoretical equations (at least a page of text would be required); instead, it is sufficient for our purposes to list the variable factors that can influence $\langle 1/T_1 \rangle$. These are: (*1*) magnetic field strength, (*2*) carbon–hydrogen internuclear distances, (*3*) orientations of C-H internuclear vectors with respect to rotational axes, (*4*) correlation times for independent modes of motion, and (*5*) spectral-density functions[12]. We have determined experimentally that field effects are insignificant for peaks 2–9, and they have been averaged to a common basis for peaks 10–12. Therefore, the differences observed among the $\langle 1/T_1 \rangle$ values could be caused by any of the other four factors.

To a good approximation, the relaxation rate, $1/T_1$, is directly proportional to Σr_{CH}^{-6}, where r_{CH} is the carbon–hydrogen internuclear distance, and the summation runs over all C-H distances within the molecule. The sum is dominated by the distance to directly bonded hydrogen atoms; but hydrogen atoms as far as two bonds removed make significant contributions. We have computed Σr_{CH}^{-6} for each carbon atom, using nuclear coordinates determined by neutron diffraction[2]. Results show that ring-carbon Σr_{CH}^{-6} values differ from their average by less than $\pm 2\%$, that is, by less than the experimental-error limits on $\langle 1/T_1 \rangle$. This means that C-H internuclear distances cannot be responsible for much of the $\langle 1/T_1 \rangle$ variation

observed in peaks 2–9. Hydroxymethyl groups have two directly bonded hydrogen atoms (*vs.* one for the ring-carbon atoms). Their Σr_{CH}^{-6} sums are approximately twice as large as those of ring-carbon atoms; therefore, we anticipate normalized relaxation rates of ~2.0 for peaks 10–12.

Relaxation rates depend on the orientations of C-H vectors with respect to the axes of rotational motion. In practice we need only consider directly bonded carbon–hydrogen atom pairs. Motion about an axis perpendicular to the C-H bond produces the maximum relaxation rate, whereas motion about an axis exactly parallel to the bond has no effect. Therefore, if the molecular conformation is rigid, and if each C-H bond-vector points in a different direction, each carbon atom responds to motions about a different molecular axis. The faster the motion, the smaller the $\langle 1/T_1 \rangle$ value.

Examination of the crystal structure[1,2] reveals that sucrose is nonspherical (molecular dimensions are 1.0 × 0.8 × 0.4 nm) and that its long axis approximately parallels the crystallographic *b* axis. Also, two sets of ring carbon atoms may be distinguished by their C-H bond orientations. Four of the C-H bonds in sucrose (to ring carbons* 2^g, 3^g, 4^g, and 5^g) lie nearly parallel to each other and almost perpendicular to the long axis of the molecule, that is, all four bonds lie within 3° of their vector sum, and all four bonds make angles of 87° ±2° to the *b* axis. Another set of four C-H bonds (to carbons 1^g, 3^f, 4^f, and 5^f) are far from parallel to each other, but they describe approximately a cone that has its axis parallel to the long axis, so that the four bonds all make angles of 27° ±10° to the *b* axis.

We may expect the most-rapid rotational motion of sucrose to be about the long axis. Therefore, the two sets of ring-carbon atoms should exhibit different relaxation times as sucrose tumbles anisotropically. Data in Table I confirm that ring-carbon atoms having C-H bonds perpendicular to the long axis (peaks 6–9) have shorter effective correlation-times (that is, smaller $\langle 1/T_1 \rangle$ values, average $\langle 1/T_1 \rangle = 0.97$) than those with bonds nearly parallel to the axis (peaks 2–5, average $\langle 1/T_1 \rangle = 1.03$). This pattern of effective correlation-times is evidence that the conformation of aqueous sucrose is similar to that of the crystal.

When a rigid object rotates anisotropically in solution, all of its motions have the same activation energy, namely, that for solvent viscosity. It is unlikely that all of the motions of a flexible object (including rotations and internal motions) would have identical activation energies. The observation of distinctly different $\langle 1/T_1 \rangle$ values shows that the different carbon atoms experience different effective correlation-times, and the lack of temperature dependence demonstrates that all ring-carbon correlation times have the same activation energies. We conclude from this argument that the conformation of aqueous sucrose is rather rigid.

The hydroxymethyl groups exhibit smaller $\langle 1/T_1 \rangle$ values than expected (<2.0) because their effective correlation-times are shortened by internal rotation. Table

*The superscripts g and f denote carbon atoms at the numbered positions on the glucose and fructose moieties, respectively.

I shows that C-6^f (peak 10) has the greatest motional freedom (smallest $\langle 1/T_1 \rangle$), whereas C-1^f and C-6^g exhibit considerably less motion. Table I also shows that the activation energy for internal rotation is greatest at C-1^f. As the solution is heated from the 2–20° range to 60–80°, the $\langle 1/T_1 \rangle$ value for peak 11 decreases by <4% (barely a significant amount), whereas the decrease for peaks 10 and 12 is definitely significant at 14 and 12%, respectively. These data demonstrate that the hydroxymethyl groups have different rates and activation energies for internal rotation.

Our results are consistent with hard-sphere calculations performed by Bock and Lemieux[6], whose results showed that the major difference between aqueous sucrose and the crystal conformation is the loss of one internal H-bond (from O-6^f to O-5^g) in the solution structure and a slightly different conformation of the furanoid ring. According to the calculations, aqueous sucrose is rather rigid, O-1^f participates in an intramolecular H-bond so that internal rotation is greatly hindered at C-1^f, internal rotation is sterically hindered at C-6^g, and rotation at C-6^f is not significantly restricted. Bock and Lemieux[6] used n.m.r. data to support their model calculations, but they did not measure temperature or concentration effects.

Our results do not agree with those of Mathlouthi *et al.*[3–5] who have suggested that the conformation of sucrose is concentration-dependent. Our data show that $\langle 1/T_1 \rangle$ values are independent of concentration over the range from 0.10 to 1.0M; this demonstrates that neither the overall molecular dimensions nor the pattern of internal motions varies with concentration.

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