near the cross-links are slow and/or anisotropic.[36] The other component behaves much like a linear polymer in a solution of similar concentration. Deuterium NMR studies of backbone reorientation in poly(isopropyl acrylate)-$d_1$/chloroform (1% cross-linked) gels[37] yielded results for $T_1$ and $T_2$ that are virtually the same as the solution results.[38] The deuterium NMR results are perhaps the most straightforward to interpret since the deuteron was shown not to be affected by dipolar (or quadrupolar) coupling as the proton and perhaps carbon-13 results are.[36]

Our current diffusion data are basically in agreement with the above results, showing that translational as well as rotational motions in the polymer beads are determined by the degree of swelling. In addition, for the majority of polymer segments, the polymer mobility in solutions and gels is similar. Since solvent diffusion is related to the segmental motions of the polymer,[26] the diffusion results for both solutions and cross-linked beads are also similar. This would not necessarily be true in very highly cross-linked systems.

(36) English, A. D.; Dybowski, C. R. *Macromolecules* **1984**, *17*, 446-449.
(37) Durairaj, B.; Blum, F. D., to be published.
(38) Blum, F. D.; Durairaj, B.; Padmanabhan, A. S. *Macromolecules* **1984**, *17*, 2837-2846.

## Conclusions

It has been shown that it is possible to measure the translational self-diffusion coefficients for solvents inside a swollen polymer bead. To the best of our knowledge this is the first direct measurement of this kind in this type of system. Such an experiment is possible because the PGSE NMR experiment is sensitive over a distance scale that is small compared to the size of the bead. In contrast, other techniques, such as radioisotope labeling, are often complicated by contributions from bulk solvent in equilibrium with the swollen gel phase. It was also demonstrated that the self-diffusion coefficients of solvent within the bead could be predicted with accuracy with the swell ratio and diffusion data from polymer solutions. These results will be useful in areas of research where swollen polymer beads are used.

**Acknowledgment.** We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society (F.D.B.), the Research Corporation (F.D.B.), the E. I. du Pont deNemours and Co., Marshall Laboratory (S.P.), and the National Science Foundation (W.T.F.) for their financial support.

**Registry No.** Polystyrene, 9003-53-6; Poly(styrene co-vinyl benzene), 9003-70-7; Toluene, 108-88-3.

# Structural Studies of Carbohydrates by Deuterium NMR: Sucrose

**P. M. Tyrell and J. H. Prestegard***

*Contribution from the Department of Chemistry, Yale University, New Haven, Connecticut 06511.* *Received November 14, 1985*

**Abstract:** Sucrose has been catalytically deuterated at several positions in the fructose and glucose rings. Deuterium NMR spectra of the labeled molecule in an ordered potassium laurate liquid crystal have been assigned on the basis of spectroscopic properties and differential rates of deuterium incorporation. Quadrupole splittings measured for various sites have been used to deduce orientational and conformational preferences for the molecule.

Carbohydrates play important structural and functional roles at the surface of biological membranes.[1-3] They function as receptors for a variety of hormones and seem to play critical roles in cell recognition and differentiation. Prerequisite to understanding their function is a molecular level description of the conformational and associational properties of these molecules when at or near membrane–water interfaces. The association of carbohydrates with membrane surfaces has received some recent attention,[4] but there is very little information relating to preferred orientation and conformation when near membrane interfaces.[5]

Recently we applied deuterium NMR to the analysis of orientational preferences of a simple deuterium-labeled monosaccharide in a liquid-crystal medium consisting of an aqueous dispersion of potassium laurate micelles.[6] While the micelle surfaces bear only a distant relationship to surfaces of biological membranes and a monosaccharide is structurally very simple compared to the oligosaccharide moieties found at membrane surfaces, the methodology appeared to offer substantial promise for the structural investigation of more complex systems. It was clear from the initial work, however, that extension to more complex systems would require both an improved method for obtaining deuterium-labeled molecules and a stepwise exploration of the spectral properties of larger saccharides. We present here an application of deuterium NMR to an orientational analysis of the simple disaccharide, sucrose, dissolved in a potassium laurate liquid crystal. The introduction of a general means of deuterium-labeling oligosaccharide moieties, and the utilization of labeling rates as a spectral assignment tool greatly enhance the potential of the methodology for application to more complex systems.

The use of deuterium NMR as a probe of orientational properties is based on the extraction and analysis of quadrupole splittings for deuteriums located in a number of specific carbon–deuterium bonds. The methods stem from the successful application of deuterium NMR to the analysis of conformational and dynamical properties of lipids in biological membranes.[7,9] They differ from most previous studies in that magnetic field ordered liquid crystals are employed to improve spectral resolution and orientational definition.[8,10,11]

(1) Ketis, N. V.; Grant, C. W. M. *Biochim. Biophys. Acta* **1982**, *689*, 194-202.
(2) McCarry, R. C.; Anderson, R.; Singhal, S. K. *Cell. Immunol.* **1982**, *71*, 293-302.
(3) Firon, N.; Ofek, I.; Sharon, N. *Carbohydr. Res.* **1983**, *120*, 235-245.
(4) Crowe, J. H.; Whiltam, M. A.; Chapman, D.; Crowe, L. M. *Biochim. Biophys. Acta* **1984**, *769*, 151-159.
(5) Skarjune, R.; Oldfield, E. *Biochemistry 1982*, *21*, 3154-3160.
(6) Prestegard, J. H.; Miner, V. W.; Tyrell, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 7192-7196.
(7) Blume, A.; Griffin, R. G. *Biochemistry* **1982**, *21*, 6230-6242.
(8) Dufourc, E. J.; Parish, E. J.; Chitrakorn, S.; Smith, I. C. P. *Biochemistry* **1984**, *23*, 6062-6071.
(9) Stockton, G. W.; Polnoszek, C. F.; Tulloch, A. P.; Hasan, F.; Smith, I. C. P. *Biochemistry* **1976**, *15*, 954-966.

0002-7863/86/1508-3990$01.50/0 

In a liquid crystal, ordered in a high magnetic field, each deuterium gives rise to a quadrupole doublet. The magnitude of the doublet splitting depends on an orientational factor describing the direction of the C–D bond in a motional averaging frame, the magnitude of the quadrupole coupling tensor elements for the deuterium, and a series of order parameters describing the nature of the motional averaging that occurs in the liquid-crystal environment.[10,12] If we make the assumption that the motion executed is approximately axially symmetric, and if we assume that all C–D bonds have sufficiently similar and axially symmetric electron distributions to be described by a single coupling constant, 170 kHz, the equations relating quadrupole splittings to molecular properties greatly simplify. In fact, they are given by the product of $^3/_2$ the coupling constant, an order parameter, $S$, and a geometry factor, $(1 - 3\cos 2\theta)/2$, in which the angle, $\theta$, is the angle between a C–D bond and the axis of motional averaging (director axis). The inverse equation, given in terms of a measured quadrupole splitting, is unfortunately not a single valued function. However, in cases where splitting can be measured for several non-colinear C–D bonds of known relative orientation, a unique orientation of the motional averaging axis can be found in a molecular frame. The position of this axis in a molecule can be interpreted in terms of preferred orientations imposed on the molecule by its environment.

Sucrose is a disaccharide consisting of a glucose 1–2 linked to a fructose by a glycosidic bond. The molecule has been extensively investigated by a number of techniques, and a crystal structure exists from which relative orientations of C–D bonds may be taken.[13] Sucrose is a nonreducing sugar, which makes it a suitable candidate for the deuteration methods we employ. These are methods introduced by Koch and Stuart that employ Raney nickel as a deuterium exchange catalyst.[14] The conditions are mild, requiring aqueous solutions at neutral pH and moderate temperatures. They offer possibilities for applications to other oligosaccharide-containing molecules including glycolipids. Orientational properties of sucrose either near membranes or in liquid crystals have not been investigated. It is thus a suitable molecule for the proposed study.

## Experimental Section

Preparation of a deuterium-substituted sucrose molecule was accomplished by following the general procedure of Balza and Perlin.[15] One gram of sucrose was deuterated by reaction with 5 g of Raney nickel suspended in 60 mL of deuterium oxide at 100 °C. The reaction mixture was filtered to remove the Raney nickel, and the sucrose solution was passed through a short Chelex column. The deuterated sucrose was further purified on a Dowex ion-exchange column, $Ca^{2+}$ form.[16] It was identified by its elution properties in comparison to a nondeuterated analogue and by its negative reaction with Fehlings solution.[17] Several samples were prepared, differing only in the length of treatment with Raney nickel. Within any one deuteration reaction the time course of deuterium exchange follows a simple monotonic function for each site. Differences in incorporation at various sites will prove useful in making spectral assignments. Rates of deuteration from preparation to preparation were not, however, highly reproducible, presumably because of the quality of the catalyst. Thus, there may seem to be variations in deuteration levels between spectra taken of samples having similar deuteration times.

The liquid-crystal phase to be used for acquiring oriented spectra is the potassium laurate phase described by Forrest and Reeves.[18] Potassium laurate was prepared by addition of an equimolar amount of lauric acid (Eastman) to a solution of 2.5 M KOH in ethanol. The potassium laurate formed was recrystallized from ethanol. The liquid crystal phase was composed of 36% potassium laurate, 4% lauric acid, 2% KCl, and 58% $H_2O$.[18] Approximately 10 mg of deuterated sucrose in 0.04 mL of $H_2O$ was added to 1.5 mL of this phase. This is a sufficiently small quantity to preserve the basic characteristics of the liquid-crystal phase.

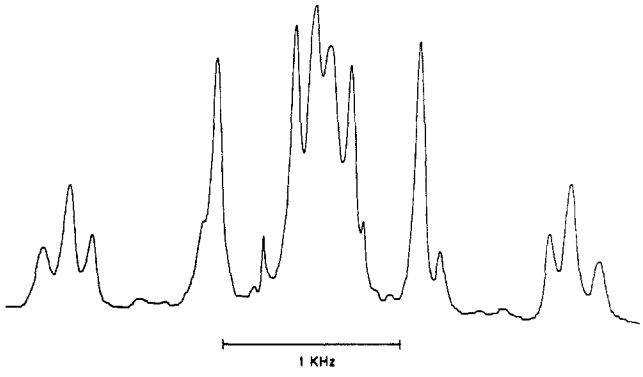


**Figure 1.** 76-HMz deuterium spectrum of deuterated sucrose (4 h of deuteraution) oriented in a potassium laurate liquid crystal (~20 mM, 313 K).

The sample in a 10-mm NMR tube was placed in a 11.7 T superconducting magnet and allowed to equilibrate at 313 K for 30 min or more. Frequently small adjustments in temperature or ionic strength were required to achieve uniform orientation. Deuterium spectra were acquired with a Bruker WM500 spectrometer at a frequency of 76.7 MHz. Each spectrum was the result of 500 scans with a 90° pulse of 40 μs, a repetition rate of 0.2, and a sweep width of 5000 Hz.

Assignment of spectra was facilitated by the separation of chemical shifts from quadrupole couplings by using 2D multiple quantum spectroscopy. Spectra were acquired with use of the pulse sequences (90–$t/2$–180–$t/2$–45–90–$t_1$–90–$t_2$) and (90–$t/2$–180–$t/2$–90–$t_1$–90–$t_2$) to collect two separate data sets for quadrature detection. Appropriate phase cycling was incorporated to achieve quadrature detection, eliminate one-quantum signals, and eliminate artifacts associated with pulse imperfections.[19,20] The first set of pulses separated by two delay periods, $t/2$, accomplished excitation of double quantum coherence. No single $t/2$ value can be found to optimize excitation of all multiple quantum coherences. One solution is to use different $t/2$ values which add as odd harmonics.[19] Therefore, data sets were collected with delay values, $t/2$, of 0.0001 and 0.0003 s and added. These values give maximum signals for doublets with quadrupole splittings equal to an odd multiple of $1/(2t)$ and zero signal for doublets with quadrupole splittings equal to an even multiple of $1/(2t)$. In both data sets the value of $t_1$ following excitation was incremented 32 times by 0.0015 s, resulting in a sweep width in the F1 dimension of +/− 333 Hz (F1 dimension). The final 90° pulse mixes double-quantum coherence into single-quantum coherence where a signal can be detected during $t_2$. Data were digitized into 2048 $t_2$ points at a rate sufficient to yield an F2 width of 6000 Hz. Total acquisition time for each data set was 4 h. The data were weighted with a shifted (45°) sinebell window function before transformation in each dimension.

Proton spectra (500 MHz) of 50 mM sucrose at 2, 4, and 8-h deuteration were acquired in order to assign deuterium spectra based on comparisons of chemical shift and resonance intensities. The spectra were the result of 16 scans, a repetition time of 0.8 s, and a sweep width of 2000 Hz. Nuclear Overhauser experiments (NOE) were also conducted to aid in the determination of probable solution conformations for the molecule. We chose to do dynamic NOEs since steady-state NOEs are subject to secondary transfers of magnetization. In these experiments radio frequency power sufficient to saturate a selected resonance in less than 0.1 s but still not perturb adjacent spectral resonances was applied for lengths of time ranging from 0.2 to 6 s. A 90° pulse was then applied, an FID accumulated, and an additional delay allowed to elapse before repetition for signal averaging. In all experiments the temperature was 303 K, and the recycling delay was 4 s. The resulting spectra were integrated to determine the magnitude of the NOE.

## Results

Figure 1 is a deuterium spectrum of sucrose deuterated for 4 h and oriented in a potassium laurate liquid crystal. The observable resonances cover a range of 3500 Hz. The spectrum

(10) Chem, D.; Fujiara, F.; Reeve, L. *Can. J. Chem.* **1977**, *55*, 2396–2403.
(11) Poupko, R.; Luz, Z.; Zimmerman, H. *J. Am. Chem. Soc.* **1982**, *104*, 5307–5314.
(12) Doane, J. W. *Isr. J. Chem.* **1983**, *23*, 323–378.
(13) Brown, G. M.; Levy, H. A. *Acta Crystallogr.* **1973**, *B29*, 790–797.
(14) Koch, H. J.; Stuart, R. S. *Carbohydr. Res.* **1978**, *67*, 341–348.
(15) Balza, F.; Perlin, A. S. *Carbohydr. Res.* **1982**, *107*, 270–278.
(16) Angyal, S. J.; Bethell, G. S.; Beveridge, R. J. *Carbohydr. Res.* **1979**, *73*, 9–18.
(17) Browne, C. A.; Zerban, R. W. *Sugar Analysis*; J. Wiley: New York, 1941; p 646.
(18) Forrest, B. J.; Reeves, L. W. *Chem. Rev.* **1981**, *81*, 1–19.
(19) Bodenhausen, G. *Prog. Nucl. Magn. Reson. Spectrosc.* **1981**, *14*, 137–173.
(20) Miner, V. W.; Tyrell, P. M.; Prestegard, J. H. *J. Magn. Reson.* **1983**, *55*, 438–452.

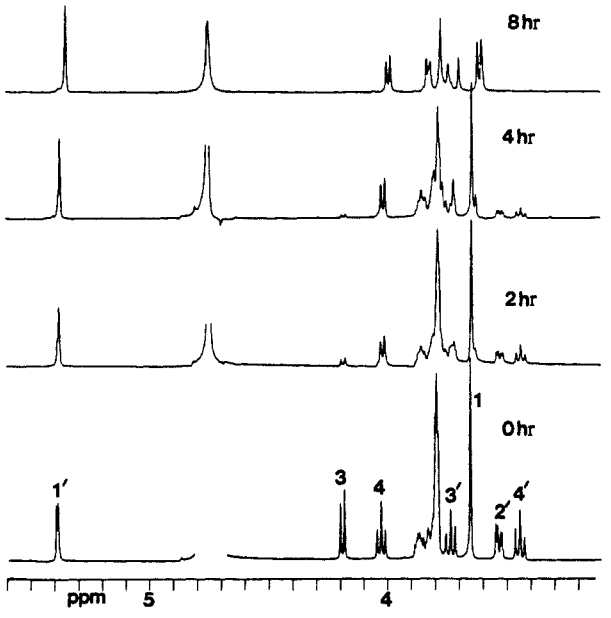


**Figure 2.** 500-MHz proton spectra of sucrose (~50 mM in D20), after various periods of deuteration. Chemical shifts are relative to DSS.

consists of a superposition of eight quadrupole doublets with different coupling constants. One of these is a sharp pair offset from the center of the spectrum of 580-Hz splitting. This is from residual HDO. There is a slight asymmetry in the spectrum due to the offset of each doublet due to chemical shift differences. This residual chemical shift difference can be used to make assignments. The doublets are also of different intensities because each site differs in its rate of deuteration with Raney nickel. The rate of increase of intensity of peaks in the deuterium spectra of oriented samples after deuteration for various periods of time can be correlated with the disappearance of intensity of assigned resonances in the proton spectra to help make assignments.

Figure 2 shows the 3–6-ppm region of the proton spectrum of sucrose along with spectra after deuteration with Raney nickel for periods of 2, 4, and 8 h. The spectrum was assigned by using the work of Bock and Lemieux[21] and was confirmed in our laboratory by using 2D scalar coupling correlated spectroscopy. From the reduction in peak amplitudes, as a function of deuteration time, it is clear that significant deuteration has occurred at several sites. Raney nickel is expected to catalyze exchange of hydrogen for deuterium at carbons bearing hydroxyls. The reaction apparently proceeds by a dehydrogenation–redeuteration process in which the sucrose molecule does not undergo racemization at exchanging sites because it is tightly bound to the catalyst.[15] In sucrose, which is composed of glucose and fructose moieties, C-2′, C-3′, C-4′, C-6′, C-1, C-3, C-4, and C-6 all bear hydroxyls and are potential sites of deuterium exchange. However, steric interactions profoundly influence the rates of exchange. It has been shown with glucose, for example, that the favored interactions with Raney nickel result in rapid deuteration of carbons 2 and 4 and slow deuteration of C-3. Glucose when in a 1–2 linkage apparently is still dominated by these intraring restrictions. The single highly deuterated site in fructose may be due to a combination of intra- and interring effects. In our experiments, reaction of sucrose with Raney nickel for even short periods of time resulted in nearly complete deuteration of H-2 and H-4 in the glucose ring and complete deuteration of H-3 in the fructose ring.

For the more slowly exchanging resonances, deuteration rates were studied in more detail by integrating residual proton resonances in samples exposed to the deuteration catalyst for periods of time from 1 to 6 h. Results are summarized in Table I. After 6 h, C-4 and C-3′ are deuterated 36% and 22%, respectively. The 1 position of the fructose ring, which represents 2 protons, is 48% deuterated at this time. The proton spectrum of residual protonated molecules shows two resonances of comparable intensity near the original H-1 chemical shift. One is near the original resonance and represents doubly protonated sites. The other is presumably offset by an isotope shift because of deuteration at one site. The existence of these two resonances at a total deuteration level of 50% suggests nearly equal exchange at H-1a and H-1b sites. Integration of resonances near 3.8 ppm, assigned to 5, 5′, 6, and 6′, shows the equivalent of 3.5 protons to have exchanged. Since 5 and 5′ are not hydroxylated, this must mean nearly complete deuteration at 6 and 6′ sites. In summary, the 3 proton exchanges most rapidly, the 2′ and 4′ protons exchange very rapidly, the four 6 protons exchange rapidly, and the 1, 4, and 3′ protons exchange slowly.

These differential rates can be correlated with the relative intensities of various quadrupole doublets in deuterium spectra of samples exposed to catalyst for varying lengths of time.

Figure 3 shows the time course of deuteration as observed in deuterium spectra. The doublet assigned to HDO varies in intensity, and splittings scale differently in various spectra due to variations in sample composition, but relative intensities of sucrose doublets can clearly be compared from spectrum to spectrum. The doublet labeled D deuterates most rapidly. Doublets with small splittings, labeled E and F, are next most rapidly deuterated. These are followed by B which is in turn followed by A and C.

(21) Bock, K.; Lemieux, R. U. *Carbohyd. Res.* **1982**, *100*, 63–74.

**Table I.** Rates of Sucrose Deuteration Determined by Changes in Intensities in $^1$H NMR Spectra

| time, h | deuteration site | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 4 | 5,5′,6,6′ | 3′ | 1 | 2′ | 4′ |
| 0.0 | 0.93 | 0.91 | 5.88 | 1.06 | 1.90 | 0.94 | 0.88 |
| 1.0 | 0.69 | 0.95 | 5.49 | 1.26 | 2.28 | 0.97 | 0.88 |
| 1.5 | 0.13 | 0.84 | 5.39 | 1.21 | 2.14 | 0.31 | 0.35 |
| 3.5 | 0.16 | 0.80 | 5.52 | 0.94 | 1.79 | 0.43 | 0.46 |
| 4.0 | 0.07 | 0.75 | 4.80 | 0.77 | 1.65 | 0.27 | 0.24 |
| 6.0 | 0.03 | 0.64 | 2.40 | 0.78 | 1.03 | 0.01 | 0.01 |

[a] Numbers are integrated intensities normalized to the area of the H-1′ resonance on the glucose ring.

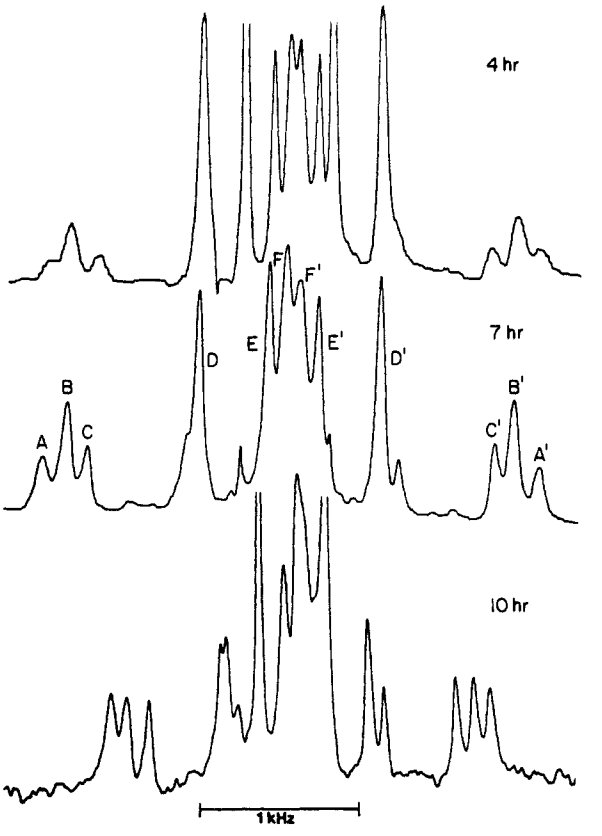


**Figure 3.** 76-MHz deuterium spectra of sucrose (~20 mM in a potassium laurate liquid crystal) after various periods of deuteration. Letters indicate pairing of resonances into quadrupole doublets.

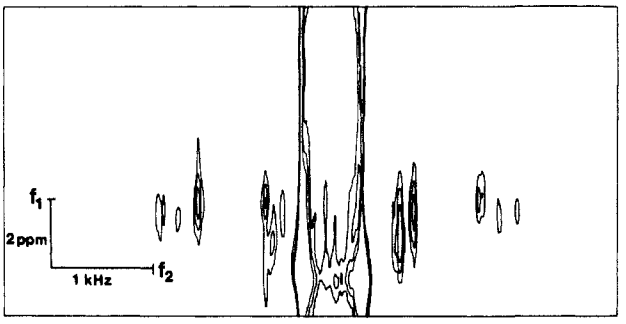


**Figure 4.** Two-dimensional contour map of a multiple quantum spectrum of deuterated sucrose (~20 mM in a potassium laurate liquid crystal). The vertical dimension has been scaled to read in normal chemical shifts. The horizontal dimension shows both chemical shift and quadrupole coupling affects.

Doublets appearing just to either side of D are very slowly labeled. This suggests a correlation of D with 3, E and F with 2′ and 4′, A, B, and C with the 6 protons, and some combination of 1a, 1b, 4, and 3′ with the peaks to either side of D.

The chemical shifts of doublets in the deuterium spectrum also provide a useful means of furthering assignments of deuterium spectra. The chemical shifts, because they arise from the electronic structure of the molecules, are nearly identical for deuterium and proton spectra. The chemical shift dispersion in deuterium spectra, however, is one-sixth that in proton spectra and deuterium lines are broader, making resolution of chemical shifts for different sites difficult. Deuterium double quantum spectroscopy can aid in separating chemical shift offsets from quadrupole coupling.[20] Quadrupole interactions contribute equally to perturbation of $m = +/-1$ spin states of deuterium. Therefore, a double quantum transition from $m = 1$ to $-1$ is independent of quadrupole splitting. Moreover, chemical shifts are scaled up by a factor of 2 from their single quantum values. The improved resolution makes it possible to separate and assign peaks which were nearly overlapping in the one-quantum spectrum.

A 2D multiple quantum spectrum of deuterated sucrose in a liquid crystal is shown in Figure 4. Only chemical shift information is displayed in the F1 dimension. Both chemical shift and coupling information are displayed in the F2 dimension. The most prominent feature is an intense pair of contours far downfield in F1 which is assigned to residual deuterium in HDO. The remainder of the contours arise from sugar deuterons. On the basis of proton chemical shift assignments, one would expect the most downfield of the deuterated sugar sites to belong to C-3. A pair of downfield contours is clearly seen in Figure 4 with a splitting of 1223 Hz. This corresponds to the D doublet which was suggested to belong to C-3 on the basis of deuteration rates. Of the remaining deuterated sites, only glucose H-2 and H-4 are sufficiently distinct in chemical shift to make assignments. On this basis, the farthest upfield pair of contours, which is nearly obscured by wings from the water contours and which can be located near the center of the contour map with a splitting of 348 Hz, is assigned to H-2′ or H-4′. This quadrupole pair corresponds to the intense E pair in the 1D spectrum. Again this corresponds to a rapidly deuterated pair and assignment is in agreement with suggested assignments based on deuteration rates. Unfortunately, assignment to H-2′ or H-4′ remains ambiguous on the basis of both shift and labeling rates. The other resolved sets of contours in Figure 4 fall at intermediate chemical shifts, which is consistent with their association with 6, 1, 4, and 3′ sites. Splittings and proposed assignments are summarized in Table II. These values will be useful in verifying any proposed structural model.

Ideally, verification of structure and determination of orientational preference requires unambiguous assignment of a sufficient number of quadrupole splittings to rigid C–D bonds of known relative orientation to eliminate order parameters and deal with the multivalued nature of the $((1 - 3\cos^2\theta)$ function. Two assignments would be enough to restrict conformational and orientational possibilities for an axially symmetric system, but three or more assignments are usually needed to identify a unique

**Table II.** Assignments of Quadrupole Splitting to Specific C–D Bonds in Sucrose

| splitting | C–D bond |
|---|---|
| 3347, 3064, 2755 | 6a, 6b, 6a, 6b |
| 1411 | 1a, 1b or 4 |
| 1223 | 3 |
| 1130 | 1a, 1b or 4 |
| 348, 78 | 2′, 4′ |

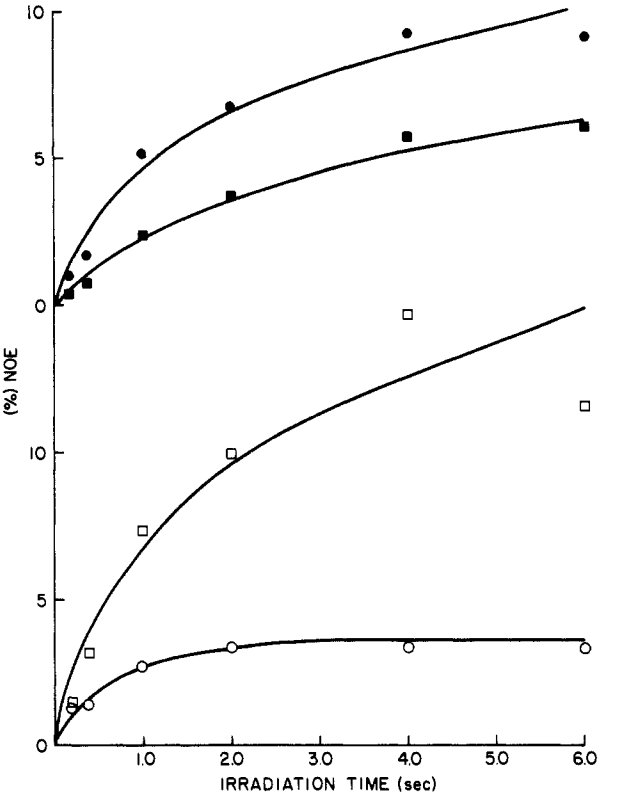


**Figure 5.** Nuclear Overhauser enhancements observed after various periods of selective presaturation. Sample conditions are ~50 mM sucrose in D20, 303 K, 500 MHz. Open symbols correspond to irradiation of the anomeric glucose proton (H-1′). Filled symbols correspond to irradiation of the fructose H-1 protons. The protons observed in each case are as follows: ●, H-1′; ■, H-3; ○, H-1; □, H-2′.

orientation. In glucose, 2′ and 4′ are nearly colinear so it is necessary to include deuterons from the fructose ring to get a sufficiently large set of data. However, we cannot necessarily assume these vectors on glucose and fructose to be rigidly fixed relative to one another as they exist in the crystal structure because they are separated by a potentially rotatable glycosidic bond. Fortunately, the mobility and geometry of sucrose can be investigated in solution by NOE experiments. These experiments allow examination of interproton distances, including those which are sensitive to rotation of glycosidic bonds. This methodology has been applied to sucrose in the past.[21] We have repeated some of that work to more thoroughly quantitate distances and to assess whether the crystal structure[13] is a good approximation of the solution structure.

Dynamic NOE experiments, in which the well-resolved H-1 resonance is irradiated and the magnetization enhancement of H-3 and H-1′ is observed, or in which H-1′ is irradiated and enhancement of H-2′ and H-1 is observed, are presented in Figure 5. At early points in the dynamic NOE experiment, the buildup of magnetization can be correctly described by using a sum of pairwise interactions. Fitting our data to equations which consider only pairwise interactions enables us to extract a cross-relaxation constant for any pair of interactions. All cross-relaxation constants are related to one another by the inverse sixth power ratios of their

internuclear distances, provided all interactions are modulated by the same isotropic motion. In molecules where one or more pairs of atoms is at a fixed, well-known distance, other unknown distances can be calculated with the following equation.

$$(r_1/r_2)^6 = \mathrm{NOE}_2/\mathrm{NOE}_1$$

Implicit in this equation is the existence of a single conformational state. Since we plan to use it only to test the validity of the crystal structure, this additional assumption is justified.

Using the H-1′–H-2′ distance of 0.24 nm as an intraring calibration since it is a fixed distance, we calculated the H-1′–H-1 distance to be 0.23 nm. This has been calculated as if a single proton contributed to the NOE. There are actually two protons on C-1, but since there is a $1/r^6$ dependence, the proton at the longer distance makes a minimal contribution. We can therefore compare the 0.23-nm distance in the solution structure to the distance between H-1′ and the closer H-1 in the crystal structure. Here the H-1–H-1′ pair is 0.22 nm apart. The crystal structure distance is 0.01 nm shorter than observed but still shows good agreement. NOEs between H-1 and H-3 on fructose also indicate that the crystal structure is a good model. The crystal structure distance for H-1–H-3 is 0.24 nm while the observed distance is 0.26 nm. The slightly longer distances calculated from NOEs could arise from some small internal bond oscillations. Rapid rotational motion about the C-1–C-2 fructose geometry shown in the crystal structure can, for example, be shown to reduce NOEs and therefore lead to longer measured distances. On the basis of the above distances and their correlation with the crystal structure, we will assume vectors arising from C–D bonds on different sugar rings have fixed relative orientations and will use their measured quadrupole coupling in an attempt to analyze a preferred sucrose orientation in a liquid crystal.

## Discussion

Analysis of the data presented above can be accomplished through an iterative search for orientations and order parameters that reproduce observed quadrupole splittings. A program has been written and previously described that carries out this search.[6] As discussed above, we would choose to input at least three quadrupole splittings. The obvious candidates are those for $H_{2'}$, $H_{4'}$, and $H_3$ sites. $H_3$ is definitely assigned and $H_2$ and $H_4$ assignments would require examination of only a single permutation. Unfortunately, $H_{2'}$ and $H_{4'}$ vectors are nearly colinear and inputing this combination of vectors produces a large set of possible orientations.

Among other possible vectors to use, $H_{3'}$ is also nearly parallel to $H_{2'}$ and $H_{4'}$, and the $H_6$ and $H_{6'}$ vectors are not necessarily rigidly fixed relative to the others because of possible rotation about exocyclic carbon–carbon bonds. $H_4$ is a good choice and $H_{1a}$ and $H_{1b}$ are good choices because the nuclear Overhauser data offer independent evidence that these vectors are predominantly in directions found in the crystal structure. $H_{1a}$, $H_{1b}$, and $H_4$ are slowly deuterating sites that could be assigned to the low-amplitude doublets with splittings of 1411 and 1130 Hz. $H_{3'}$ is also slowly deuterating but because of its being nearly parallel to $H_{2'}$ and $H_{4'}$ it should have a splitting of a few hundred rather than a thousand or more hertz. This leaves six ways of assigning the 1411 and 1130 lines and two ways of assigning the 78 and 348 lines to $H_{2'}$ and $H_{4'}$. Thus, we have twelve possibilities to examine. Asking that all splittings be reproduced to within 90 Hz, we find only a single group of closely spaced orientations for the sucrose molecule. The calculated and observed splittings for the best director orientation are compared in Table III.

Although, not included in the fit, splittings for other labeled sites are presented in the table. The $H_{3'}$ site is predicted to have a splitting of approximately 100 Hz. This would be obscured by the intense $H_{2'}$ lines. $H_4$ is predicted to have a splitting of a little over 1200 Hz. This would be obscured by the intense $H_3$ line at a 1223-Hz splitting. The $H_6$ and $H_{6'}$ splittings, calculated by using orientations found in the crystal structure, depart significantly from the positions of any observed lines. The disagreement is not unexpected based on the possible rotational freedom of these groups. In fact there is evidence that the glucose-6-hydroxyl does not adopt the same conformation in solution as that found in the crystal.[21] If we assume, for the fructose-6-hydroxymethylene, that rapid equilibration among three equally populated rotational isomers takes place, we would predict splittings for the 6a and 6b deuterons of 2900 Hz. This is in the midst of the three observed splittings between 3400 and 2700 Hz. Thus we feel the total agreement between predicted and observed splittings is excellent.

**Table III.** Comparison of Calculated and Observed Quadrupole Splittings for Sucrose with Director

| site | calcd splitting, Hz | exptl splitting, Hz | error, Hz |
|---|---|---|---|
| 1b[a] | 1411 | 1411 | 0 |
| 4 | 1281 | 1223 | 58 |
| 3[a] | 1235 | 1223 | 22 |
| 1a[a] | 1148 | 1130 | 18 |
| 4′[a] | 313 | 348 | 35 |
| 3′ | 134 | 78 | 56 |
| 2′[a] | 83 | 78 | 5 |
| 6a | 5600 | | |
| 6′a | 5000 | 3347 | |
| 6b | 3700 | 3064 | |
| 6′b | 3000 | 2755 | |

[a] Data used in initial fit.

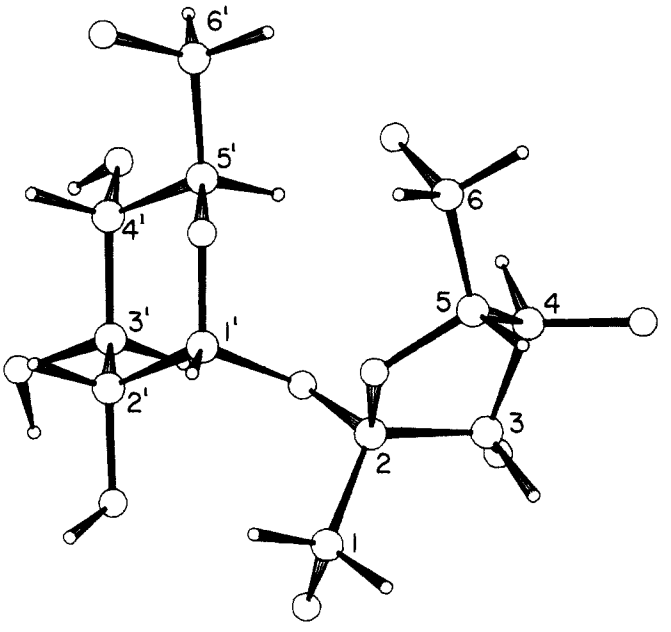


**Figure 6.** Structure of sucrose showing the preferred orientation if the magnetic field were perpendicular to the plane of the paper.

If we define the directors about which axial averaging occurs in a coordinate system taken from the crystal structure, we find a vector of -0.357, 0.713, -0.278. Figure 6 is drawn so that this vector is approximately perpendicular to the page. Because quadrupole splittings are insensitive to inversion, the director may point either into or out of the page.

We can define the director in a way which makes comparison with directors for other sugars a little easier. An $x$ axis is defined to be coincident with the $C_{1'}$–ring oxygen bond in the glucose moiety. A $y$ axis is defined to be perpendicular to the $C_{1'}$–O and O–$C_{5'}$ bonds, and a $z$ axis is defined to be perpendicular to $x$ and $y$. Angles for the director relative to each of the axes are then 96°, 100°, and 27°.

At this stage we cannot draw many firm conclusions from the observed orientation of the sucrose molecule. The medium is a nematic liquid crystal composed of cylindrical micelles of fatty acids oriented with their long axes parallel to the field. For an elongated molecule such as sucrose, one might expect one of the primary factors influencing orientation to be steric restraints on packing into the interstitial spaces. This would place the director more or less along the elongation direction of the sucrose molecule. This is clearly not the case. Some sort of surface association must

play a role. A very closely related micellar phase composed of disk-shaped micelles can be prepared. This would presumably have very similar surface properties but very different interstitial spaces. It might provide a reasonable test of the relative importance of steric and surface association forces.

The fact that we were able to find an orientation which, when combined with C–D bond vectors taken from the crystal structure, reproduces observed quadrupole splittings is interesting in itself. It also suggests that it is highly likely that the structure of sucrose in a liquid crystal environment is in close agreement with that found in the crystal or in simple solutions. It also suggests that the rather drastic assumption that the molecule executes an axially symmetic motion is not far from correct. This latter suggestion is actually supported by other evidence. Deuterium spectra such as those shown in Figure 3 actually show variations in quadrupole splittings due to variations in the composition of the liquid crystal or variations in the temperature at which the spectra were run. All splittings, with the possible exception of those assigned to deuterons on groups with internal motions (6 and 6′ deuterons), seem to scale with a single order parameter. If motion were not axially symmetric one would require several order parameters to describe motional averaging, and it is unlikely that all of these would respond the same way to environmental changes or have the same effect on all deuterated sites.

In addition to providing structural and motional information, the experiments illustrate some new methodology. In particular, the data illustrate the potential for catalytically labeling molecules and using the differential rates of label incorporation at different sites to make assignments. Although we were able to confirm assignment using chemical shift information in a few cases, our ability to do this may degrade with increasing line widths as we move to larger and larger molecules. The experiments, therefore, significantly enhance the promise that quadrupole coupling data on molecules in oriented media can be of use in the conformation and orientation study of more complex oligosaccharides and membrane systems.

**Acknowledgment.** This research was supported by a grant from the National Institution of Health (GM 33225) and benefited from instrumentation programs of the National Institute of General Science (GM 32243S1).

**Registry No.** Sucrose, 57-50-1.

# Powder ENDOR Spectra of *p*-Benzoquinone Anion Radical: Principal Hyperfine Tensor Components for Ring Protons and for Hydrogen-Bonded Protons

**Pádraig J. O'Malley[†] and Gerald T. Babcock***

*Contribution from the Department of Chemistry, Michigan State University, East Lansing, Michigan 48824-1322.* *Received November 18, 1985*

**Abstract:** ENDOR spectra for the immobilized *p*-benzosemiquinone anion radical ($BQ^{\bullet-}$) in disordered matrices are presented. Hyperfine interactions of the unpaired electron with three different classes of protons are apparent in the spectra and have been investigated: $\alpha$-proton, hydrogen-bonded proton, and general matrix proton. Dipolar interactions are not averaged in powder samples, and first derivative ENDOR lines are observed for $\alpha$- and hydrogen-bonded protons at frequencies which correspond to their principal hyperfine tensor values. Interpretation of the spectra has been facilitated by selective deuteration of the parent quinone and of the solvent. The *g* anisotropy of $BQ^{\bullet-}$, although weak, allows orientation selection experiments at X-band which have provided information on the axis directions for the tensor components relative to the molecular structure. Hydrogen bonding of the $BQ^{\bullet-}$ carbonyl group to the alcohol hydroxyl group of the solvent is characterized by a purely dipolar interaction exhibiting axial symmetry. The hydrogen bond direction is in the plane of the quinone ring and the O···H bond distance is calculated to be 1.6 Å. The principal hyperfine tensor components of the $\alpha$-proton interaction are shown to depend critically on the nearest neighbor carbon spin density values which cause the principal values to deviate substantially from those expected for an isolated $^{\bullet}C$–H fragment. For the unpaired electron spin density distribution in $BQ^{\bullet-}$, the $\alpha$-proton hyperfine tensor acquires approximately axial character. In the matrix region, several classes of weakly interacting protons contribute to the structured ENDOR line shape observed; orientation selection and selective deuteration have been used to resolve the origin of some of the lines in this region. The observed ENDOR band shapes for each type of proton–electron interaction indicate that the nuclear relaxation probability ($\omega_n$) is independent of orientation.

Since the first demonstration of the ENDOR response in 1956[1] it has been widely used in the analysis of free radical species in both the liquid and the solid state.[2] Recent developments in spectrometer design have demonstrated the feasibility of applying the ENDOR technique to most organic radicals in liquid solution.[3-5] The benefits of this approach are particularly obvious for complex organic molecules (e.g., the chlorophylls) where the large number of hyperfine interactions often leads to an unresolved EPR spectrum. A disadvantage with liquid solution EPR and ENDOR studies, however, is that information on the electron dipole–proton dipole interaction is lost owing to the averaging of this term by the radical tumbling in solution. One usually resorts to single-crystal studies to retrieve this information. Unfortunately, this approach is not feasible in some cases, particularly in biological systems, where only powder samples or disordered solids are available.

The ENDOR spectrum of organic radicals in disordered solids is usually complicated by the broadened nature of the ENDOR response observed.[6] In certain favorable cases, where *g* or hy-

[†] Present Address: Department of Chemistry, University of Manchester, Institute of Technology, P.O. Box 89, Manchester M60 1QD, UK.

(1) Feher, G. *Phys. Rev.* **1956**, *103*, 834.
(2) Kevan; Kispert, L. D. In *Electron Spin Double Resonance*; Wiley: New York, 1976.
(3) Mobius, K.; Frohling, W.; Lendzian, F.; Lubitz, W.; Plato, M.; Winscom, C. J. *J. Phys. Chem.* **1982**, *86*, 4491.
(4) (a) Lubitz, W.; Lendzian, F.; Mobius, K. *Chem. Phys. Lett.* **1981**, *81*, 235. (b) Kurreck, H.; Bock, M.; Bretz, N.; Elsner, M.; Krans, H.; Lubitz, W.; Muller, F.; Geissler, J.; Kroneck, P. M. *J. Am. Chem. Soc.* **1984**, *106*, 737.
(5) (a) Kurreck, H.; Kirste, B.; Lubitz, W. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 173. (b) Mobius, K.; Lubitz, W., submitted.

0002-7863/86/1508-3995$01.50/0 