oligomers will help to characterize the internal motion of these biomolecules and will provide important insights into the possible functional role of such flexibility.

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The ^{18}O Isotope Shift in ^{13}C Nuclear Magnetic Resonance Spectroscopy. 12. Position of Bond Cleavage in the Acid-Catalyzed Hydrolysis of Sucrose[1]

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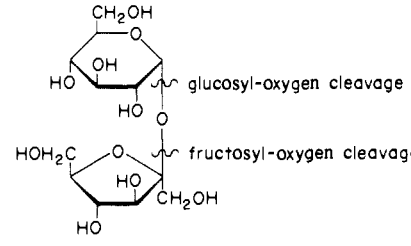
Abstract: The ^{18}O isotope-induced shift in ^{13}C NMR spectroscopy was used to elucidate the point of bond cleavage in the acid-catalyzed hydrolysis of sucrose. Sucrose was hydrolyzed in the presence of $H_2{}^{18}O$, and the incorporation of ^{18}O into the several anomeric products was simultaneously assayed as a function of time. While the equilibrium ratio of ^{18}O was detected in the fructose anomers throughout the course of the hydrolysis, the percentage incorporation of ^{18}O into the glucose anomers slowly increased to the equilibrium ratio. Independent experimental evidence was obtained with ^1H NMR; the ratio of β-glucose to α-glucose was observed to increase from an initially detected value of less than 2:3 to the equilibrium value of greater than 3:2 during the hydrolysis reaction. The data from the NMR experiments were quantitated and compared to theoretical models based on hydrolysis mechanisms involving fructosyl–oxygen bond cleavage and glucosyl–oxygen bond cleavage. The results clearly indicate fructosyl–oxygen bond cleavage under the reaction conditions employed.

The acid-catalyzed hydrolysis of sucrose has a rich history. It was perhaps the earliest catalytic reaction to be observed.[2] It was the first reaction to be studied kinetically[3] and was one of the first reactions to be studied as a function of temperature.[4] Arrhenius developed the equation bearing his name using data from early sucrose hydrolysis experiments.[5] Subsequently, debate arose concerning whether, in fact, the hydrolysis of sucrose followed Arrhenius' original formulation. In 1947 Moelwyn-Hughes summarized the results of several investigators, stating "There no longer remains any doubt that the Arrhenius energy of activation for the inversion [hydrolysis] of cane sugar shows a decrease as the temperature is raised."[6] Recently this conclusion has been vigorously challenged.[7]

There is also controversy regarding the detailed mechanism of the hydrolysis reaction. The reaction is generally considered to follow an A-1 mechanism in which a fast preequilibrium protonation is followed by a unimolecular, rate-determining heterolysis of a carbon–oxygen bond, although general acid catalysis of the hydrolysis has also been proposed.[8] The rate constant of the specific acid-catalyzed reaction has been shown to vary with sucrose concentration, the acid concentration, and the concentration of the supporting electrolyte.[9] The site of protonation is generally considered to be the bridge oxygen, although protonation of the fructosyl-ring oxygen has also been proposed.[10] Finally, the identity of the bond that cleaves in the rate-determining step to form the reaction products has not been satisfactorily demonstrated,[11] despite the several proposals that have been published (see Discussion).



The question of the site of bond cleavage in sucrose remains unresolved primarily because of uncertainties inherent in the experimental techniques previously employed. The ^{18}O isotope-induced shift in ^{13}C NMR[12] has been used to study a number of hydrolysis reactions. In favorable cases, this technique may be used to assay simultaneously the rate of hydrolysis, the position of bond cleavage, and any subsequent oxygen-exchange reactions of the products. This technique appeared to be uniquely suited to resolve the question of the site of bond cleavage in sucrose. In this paper we describe the results of our investigation using the ^{18}O isotope shift in ^{13}C NMR to elucidate the point of bond cleavage in the acid-catalyzed hydrolysis of sucrose; independent

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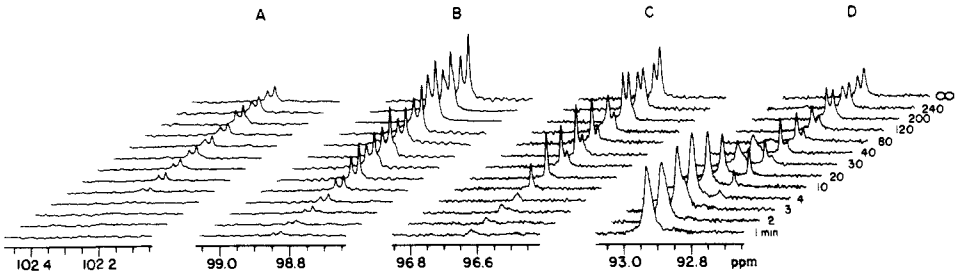


Figure 1. Hydrolysis of 0.9 M sucrose in 60% [^{18}O]water (60% $H_2{}^{18}O$, 20% ^2H_2O, 20% H_2O) plus 2 M NaCl at pH 2.9 and 80 °C followed as a function of time using ^{13}C NMR. Signals shown correspond to the anomeric carbons of (A) β-D-fructofuranose, (B) β-D-fructopyranose, (C) β-D-glucopyranose, and (D) sucrose (glucosyl C-1) and α-D-glucopyranose. The presence of ^{18}O causes an upfield shift in the ^{13}C NMR signals of approximately 0.023 ppm for the fructose anomers and approximately 0.017 ppm for the glucose anomers. The reaction time before quenching is shown at the right; the final spectrum in the series corresponds to the equilibrated product mixture. Chemical shifts are referenced to internal dioxane at 67.4 ppm.

supporting evidence was obtained through the use of ^1H NMR spectroscopy.

Experimental Section

Materials. Sucrose (99+%, gold label) and [^2H]water (99.96 atom % ^2H and 99.8 atom % ^2H) were purchased from Aldrich; deuterium chloride (99 atom % ^2H) was purchased from Stohler; and [^{18}O]water (98 atom % ^{18}O) was purchased from Merck. Glass-distilled deionized water and analytical-grade reagents were used in the preparation of the solutions.

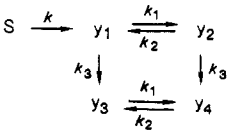
pH measurements were made with a Corning Model 130 pH meter. Since pH measurements of the 2 M NaCl solutions gave anomalous results,[13] measurements were made on solutions diluted 10:1 in H_2O. For each sample at least two such dilutions were prepared, and pH meter readings were made in duplicate; the pH meter was calibrated at ±1 pH unit of the sample pH before and after each measurement with buffer standards. Diluting and back-calculating the pH in this way gave excellent results with control solutions containing no salt.

^{13}C NMR. Weighed amounts of sucrose and NaCl were dissolved in water (60% $H_2{}^{18}O$, 20% $^2H_2{}^{16}O$, 20% $H_2{}^{16}O$) to give 6.5 mL of a 0.9 M sucrose plus a 2 M NaCl solution. The atom % ^{18}O of the water used in the hydrolysis reaction was determined by reacting 70 mg of the water with a 50% molar excess of 2,2-dimethoxypropane as described;[14] the ^{18}O content of the resulting acetone was found by ^{13}C NMR to be 60 ± 4%. Dioxane (0.1 mL) was added as a chemical shift reference. This sample was equilibrated in a circulating water bath at 80 ± 0.2 °C (the temperature of the sample was verified by a precision-grade thermometer) for 30 min before addition of 0.2 mL of a ^2HCl solution to initiate the hydrolysis reaction; the pH of the solution was 2.9. Aliquots of the solution were removed at predetermined times, immediately frozen in liquid nitrogen, and stored at −90 °C. For NMR analysis, each sample was thawed at room temperature and at least two spectra were recorded at ambient temperature (24–25 °C) with a QE-300 spectrometer fitted with a 5-mm probe and operating at 75.6 MHz. The probe temperature was calibrated by a chemical thermometer.[15] Each spectrum was collected by signal-averaging 300 transients spaced 6 s apart by using a 90° pulse width, an acquisition time of 2.3 s, a 16K block size, and a sweep width of 3600 Hz. Broad-band proton decoupling was achieved using 1 W and a MLEV decoupling sequence.[16]

^1H NMR. 50 mM Sucrose Experiment. Spectra were obtained by an NTC-200 spectrometer fitted with a 12-mm probe equilibrated at 80 ± 1 °C. (The probe temperature in the NTC-200 was calibrated with a chemical thermometer.[15]) Weighed amounts of sucrose and NaCl were dissolved in ^2H_2O (99.96 atom % ^2H) to give 5 mL of a 50 mM sucrose plus a 2 M NaCl solution. The sample was placed in the probe for a minimum of 30 min before initiating the reaction by adding a small volume of a ^2HCl solution. Single-transient spectra were collected at 30-s intervals with a 90° pulse, 7-s acquisition time, 8K block size, and a sweep width of 600 Hz. The water peak was saturated by applying 0.1 W of decoupling power during the acquisition period only.

0.9 M Sucrose Experiment. Conditions as above were used except for the following: The solution was 0.9 M sucrose plus 2 M NaCl, a 5-mm probe was used, the sample volume was 0.75 mL, the acquisition time was 2.6 s, the block size was 4K, and the sweep width was 800 Hz. The water peak was not saturated.

Scheme I



T_1 experiments of the starting material and products under the reaction conditions were performed with an inversion–recovery sequence with nine τ values and a delay time of 60 s.

Data Analysis

The FIDs from the ^{13}C NMR experiment were zero-filled twice, and a line-broadening factor of 0.3 or 0.05 Hz was applied prior to Fourier transformation. The percentages of the isotopic species of the anomeric products were estimated by averaging the results of direct peak height measurements and by spectral deconvolution of the peak areas using the CAP subroutine of the CHARM software provided with the QE-300 spectrometer.

The FIDs from the ^1H NMR experiments were zero-filled, and line-broadening factors of 0.05 Hz (0.9 M sucrose) and of 0.5 Hz (50 mM sucrose) were applied prior to Fourier transformation. Signals corresponding to the anomeric protons (H-1) of sucrose, α-glucose, and β-glucose were integrated and expressed as a percentage of total sugar.

Model for Hydrolysis of Sucrose (Theoretical Equations). If fructosyl–oxygen bond cleavage takes place, then the appearance of the different anomeric and isotopic forms of glucose can be described as in Scheme I, where S represents sucrose, y_1 and y_2 represent α- and β-glucose, respectively, prior to oxygen exchange with the solvent, and y_3 and y_4 represent α- and β-glucose after oxygen exchange with the solvent. The oxygen-exchange process (k_3) is effectively irreversible since the oxygens from y_1 and y_2 are diluted out by the bulk solvent oxygen. It is assumed that the oxygen-exchange rates are the same for both anomers[17] and that the mutarotation rates are not significantly affected by the presence of ^{18}O. The disappearance of sucrose follows simple first-order kinetics; the appearance of y_i is described by eq 1. The

$$\begin{aligned} y_1' &= kS - (k_1 + k_3)y_1 + k_2y_2 \\ y_2' &= k_1y_1 - (k_2 + k_3)y_2 \\ y_3' &= k_3y_1 - k_1y_3 + k_2y_4 \\ y_4' &= k_3y_2 + k_1y_3 - k_2y_4 \end{aligned} \quad (1)$$

solution to this nonhomogeneous series of differential equations can be obtained by applying the Laplace transform,[18] where $y_i(0) = 0$. The solutions are given in eq 2 for $i = 1$–4. The coefficients,

$$y_i(t) = c_{i1}e^{-kt} + c_{i2}e^{-(k_1+k_2)t} + c_{i3}e^{-k_3t} + c_{i4}e^{-(k_1+k_2+k_3)t} + c_{i5} \quad (2)$$

c_{i1}–c_{i5}, are listed in the Appendix. If the reaction medium has a percentage, P, of ^{18}O, then the percentage incorporation of ^{18}O into the glucose anomers at any time is given by eq 3.

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$$\alpha\text{-glucose: } Py_3(t)/[y_1(t) + y_3(t)] \quad (3)$$

$$\beta\text{-glucose: } Py_4(t)/[y_2(t) + y_4(t)]$$

Results

The incorporation of ^{18}O into the various anomeric products resulting upon the hydrolysis of sucrose in 60% [^{18}O]water is shown in Figure 1. The ^{18}O isotope-induced shift in ^{13}C NMR has been used to study the oxygen-exchange reaction at the anomeric carbons of glucose[17a] and of fructose.[17b] Consistent with those studies, the signals corresponding to the ^{18}O isotopomers are shifted upfield with respect to the ^{16}O species by approximately 0.023 ppm for the fructose anomers and 0.017 ppm for the glucose anomers. As sucrose is hydrolyzed in the solution containing [^{18}O]water, the ^{18}O content of both α-glucose and β-glucose slowly increases. This observation is consistent with an initial formation of unlabeled glucose upon hydrolysis, followed by a somewhat slower exchange with the solvent until isotopic equilibrium is reached. In contrast, throughout the reaction, the two predominant fructose anomers (β-pyranose and β-furanose) each exhibit an average ^{18}O content of 60% with a standard deviation of 2%. The final spectrum in the series (∞ in Figure 1) shows the equilibrium product mixture and demonstrates that when the hydrolysis reaction has been completed and the oxygen-exchange reactions have reached equilibrium, all species display an ^{18}O incorporation of 60%.

The results from a quantitative analysis of the spectra in Figure 1 are presented in Figure 2. The experimental percent incorporation of ^{18}O into the products is plotted as a function of time; on the same graph are shown theoretical curves for the percent incorporation of ^{18}O into the products based upon schemes involving fructosyl–oxygen bond cleavage (Figure 2A)[19] and glucosyl–oxygen bond cleavage (Figure 2B). Values for the four parameters k, k_1, k_2, and k_3 were needed to construct the theoretical curves. The pseudo-first-order rate constant, k, for the hydrolysis of sucrose was obtained from first-order plots of the intensities of several sucrose peaks and averaged to give $k \simeq 4 \times 10^{-4}$ s^{-1}. For fructosyl–oxygen bond cleavage a value for k_1 of 1×10^{-2} s^{-1} was used, which is in accord with an estimated value from available literature sources for mutarotation.[20,21] A value for k_2 was calculated from the anomerization equilibrium constant, $K_{eq} = k_1/k_2$, which for glucose was 1.5 on the basis of ^{13}C NMR peak intensities measured at reaction completion. A value for k_3 of 2×10^{-4} s^{-1} was used, which is in good agreement with an estimated value for the oxygen exchange of glucose under similar reaction conditions.[22] The theoretical curves for the glucose anomers were calculated with eq 3, and the results are shown in Figure 2A. Since the rate of anomerization is approximately 1 order of magnitude greater than the rates of hydrolysis (k) and of oxygen exchange (k_3), a rapid equilibrium is effectively established between the two anomers upon hydrolysis. As a result the theoretical curves for the two anomers coincide; the curves are sensitive primarily to the values of k and k_3 and not to k_1 and k_2.

The theoretical curves for glucosyl–oxygen bond cleavage (Figure 2B) were calculated after making the simplifying assumption that the anomerization of fructose involves only the β-pyranose and β-furanose isomers. (The α-furanose and α-pyranose forms together make up only approximately 10% of the fructose in solution,[23] a value that is consistent with the present

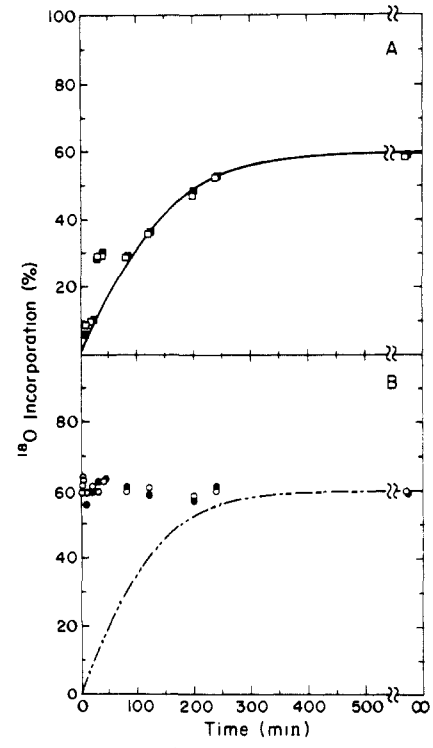

Figure 2. Incorporation of ^{18}O into the anomeric products resulting from the hydrolysis of sucrose in 60% [^{18}O]water (conditions as in Figure 1). Symbols: □, α-D-glucopyranose; ■, β-D-glucopyranose; ●, β-D-fructofuranose; and ○, β-D-fructopyranose. Calculation of the theoretical curves corresponding to fructosyl–oxygen bond cleavage (—) and glucosyl–oxygen bond cleavage (- - -) is explained in the text. Throughout the reaction, the fructose anomers displayed an average ^{18}O content of 60 ± 2%, in disagreement with the model involving glucosyl–oxygen cleavage.

results.) This assumption allows direct formulation of a scheme analogous to Scheme I where y_1 and y_2 now represent the β-furanose and β-pyranose isomers, respectively, prior to oxygen exchange, and y_3 and y_4 now represent these species subsequent to oxygen exchange. The anomerization rate k_1 was estimated from literature values[20,21] to be $k_1 \simeq 7$–8×10^{-2} s^{-1}. The equilibrium constant for fructose anomerization, $K_{eq} = k_1/k_2$, was estimated[23] to be 2 from which a value for k_2 was calculated. The maximum rate of oxygen exchange for fructose under the reaction conditions was estimated from independent measurements, extrapolated to 80 °C, to be $k_3 = 3 \times 10^{-4}$ s^{-1}.[17b] As mentioned above, since the rate of anomerization is at least 1 order of magnitude greater than the rates of hydrolysis and oxygen exchange, the values of k_1 and k_2 do not significantly affect the shapes of the curves for the different anomers. It is evident that the experimental results clearly fit a model involving fructosyl–oxygen bond cleavage but do not fit the model involving glucosyl–oxygen bond cleavage.

Since a discontinuous assay procedure was employed, it was necessary to demonstrate that no significant hydrolysis or oxygen-exchange reaction occurred during the 1–2 h in which the spectra were acquired. The aliquot quenched at 30 min was therefore allowed to remain at room temperature and the extent of reaction periodically assayed by ^{13}C NMR over a 6-week period. From these spectra first-order plots were made, which indicated a $t_{1/2} \simeq 17$ days for the hydrolysis reaction and a $t_{1/2} \simeq 50$ days for the glucose oxygen-exchange reaction.

In order to verify the major conclusion of the ^{13}C NMR experiments, independent experimental evidence was obtained by ^1H NMR spectroscopy. The ^1H NMR experiments dealt with the nonequilibrium production of anomers rather than isotopomers. Figure 3 shows representative ^1H NMR spectra acquired during the hydrolysis of sucrose in ^2H_2O under reaction conditions closely similar to those of the ^{13}C NMR experiment. The peak integrations above each spectrum show the variation in the β-glucose:α-glucose ratio during hydrolysis. The areas under all three

(19) During acquisition of the spectra for the 30- and 40-min samples, the instrument was poorly shimmed, resulting in extraneous peaks 0.017 ppm upfield of every signal in the spectrum. That this artifact resulted in an overestimate of the glucose ^{18}O content was subsequently verified for the 40-min sample: a further spectrum indicated an ^{18}O content of 17% (rather than 30%) for both anomers.

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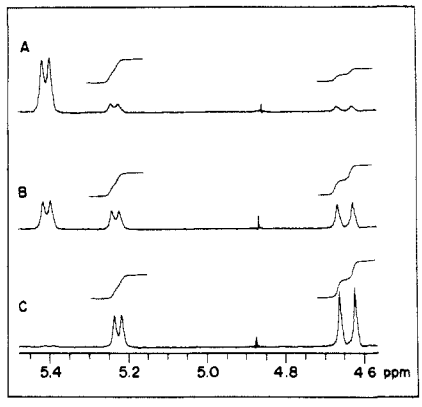

Figure 3. Examples of ^1H NMR spectra acquired during the hydrolysis of 0.9 M sucrose in ^2H_2O plus 2 M NaCl at p^{2}H 2.3 and 80 °C. The three doublets (from left to right) correspond to the anomeric protons (H-1) of sucrose, α-glucose, and β-glucose. The spectra shown were collected (A) 3.0 min, (B) 9.0 min, and (C) 39.5 min after initiating hydrolysis. The integrated peak area of β-glucose relative to α-glucose is shown above each spectrum. (The artifact at 4.87 ppm corresponds to the transmitter frequency.) Note that at early times, α-glucose is present in excess of the proportion observed at equilibrium, as expected for fructosyl–oxygen bond cleavage.

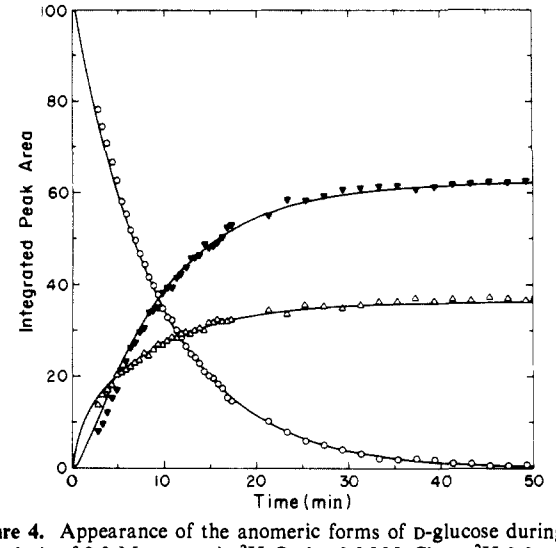

Figure 4. Appearance of the anomeric forms of D-glucose during the hydrolysis of 0.9 M sucrose in ^2H_2O plus 2 M NaCl at p^{2}H 2.3 and 80 °C. Symbols correspond to the anomeric protons (H-1) of the following: ○, sucrose; ▼, β-glucose; and △, α-glucose. Calculation of the theoretical curves for fructosyl–oxygen cleavage is described in the text.

doublets were integrated to estimate the relative proportions of sucrose, α-glucose, and β-glucose as a function of time. The results (Figure 4) demonstrate that, during the initial stages of hydrolysis, α-glucose is produced in proportions greater than that of β-glucose but that, as the reaction proceeds, the amount of β-glucose slowly increases until the equilibrium ratio is reached. The experimental data for this reaction were compared to theoretical curves calculated for fructosyl–oxygen bond cleavage (Scheme I; y_1 = α-glucose, y_2 = β-glucose, $k_3 = 0$).[24] The value of k, describing the first-order disappearance of sucrose, was estimated from a first-order plot of the sucrose peak intensities to be 1.8×10^{-3} s^{-1}. A value for $k_1 = 9 \times 10^{-3}$ s^{-1} was used, which is in good agreement with extrapolations from literature values.[20,21] The ratio $k_1/k_2 = K_{eq} \simeq 1.7$ was obtained from the equilibrated reaction mixture and used to calculate k_2.

Although the concentration of sucrose in the above experiments was relatively high (0.9 M), no spurious signals corresponding to unexpected products were observed in any of the spectra. Still, to establish that the concentration did not influence the nature of the reaction, we carried out a ^1H NMR experiment under similar reaction conditions but using an 18-fold lower initial concentration of sucrose (50 mM). The results (not shown) agreed very well with the reaction at higher sucrose concentration: a plot of the peak intensities as a function of time showed the same trends demonstrated in Figure 4, and the ratio of β-glucose:α-glucose in both reactions increased from an initially observed value of less than 2:3 to an equilibrium value of greater than 3:2. Quantitation of the data was more difficult due to the significant decrease in the signal-to-noise ratio, but the nonequilibrium production of α-glucose was again clearly demonstrated.

Finally, quantitative NMR requires that acquisition pulses be spaced far enough apart to allow the nuclear energy level populations to reestablish equilibrium. A delay time of 5 times the longest T_1 is recommended since this allows for 99% recovery. The peak with the longest T_1 time in both ^1H NMR experiments was that of α-glucose. For the 0.9 M sucrose experiment $5T_1$ delays were used between acquisition pulses, while $3.5T_1$ delays (corresponding to 97% recovery) were used for the 50 mM sucrose experiment. The validity of quantitative applications using the ^{18}O isotope shift in ^{13}C NMR has been demonstrated.[12,17a,25]

Discussion

In 1967 BeMiller pointed out that a mechanistic interpretation of the results from the sucrose hydrolysis studies to date was problematic.[26] While the limited data on furanoside hydrolysis favored an A-2 mechanism, the bulk of the evidence on glucopyranoside hydrolysis indicated an A-1 scheme. Since sucrose was hydrolyzed with the activation energy and entropy values of furanosides and yet was thought to utilize an A-1 mechanism, BeMiller suggested that an A-1 scheme involving fructosyl–oxygen bond cleavage was plausible but concluded that "more experimentation needs to be done".

Since then, several proposals have been made concerning the point of bond cleavage in sucrose hydrolysis. Following BeMiller's lead, some have proposed fructosyl–oxygen cleavage on the basis of an analysis of rates and energy parameters.[10,27] Others have suggested solely glucosyl–oxygen cleavage.[28] The only previous ^{18}O-labeling study concluded that both fructosyl–oxygen and glucosyl–oxygen scission took place.[29] (The results of this latter study, however, were ambiguous, and the heterogeneous reaction system that was used has been criticized as not being comparable to previous studies.[27b]) Textbooks also present varied schemes for the point of bond cleavage. One text suggests simultaneous cleavage of both bonds,[30] while another presents a detailed reaction mechanism involving glucosyl–oxygen bond fission.[31] Finally, one often-encountered scheme depicts sucrose (β-D-fructofuranosyl-α-D-glucopyranoside) being hydrolyzed directly to β-D-fructofuranose and α-D-glucopyranose.[32] This scheme makes the dubious implication either that these two species are the initial reaction products or that they are the major reaction products.

In contrast to the other common disaccharides, both monosaccharide units of sucrose are linked via their anomeric carbon atoms. Upon hydrolysis each monosaccharide is free to undergo

(24) The data in Figure 4 are shifted slightly to the right relative to the theoretical curves. This may be due to the slight cooling that inevitably occurs when the sample is transferred into the NMR probe after initiating the reaction.

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anomerization. Since this second reaction is fast relative to hydrolysis, it is in general very difficult to identify the initial reaction products. In fact, Kubler searched for similar initial products in his studies modeling the hydrolysis of sucrose with simple aldal acetals but could find none.[11] Extending his studies in this area Kubler recently demonstrated how a gas chromatography method could be used to observe the initial products of the invertase-catalyzed hydrolysis of sucrose.[33] As he points out, however, the rapid anomerization reactions of fructose and glucose in acidic solution prevented the application of this method to a conclusive demonstration of the initial products in the acid-catalyzed hydrolysis of sucrose. In the absence of direct evidence about the initial products, the point of bond cleavage (and thus the detailed mechanism of the hydrolysis of sucrose) has remained unresolved.

In order to make meaningful observations of the *initial* products upon hydrolysis of sucrose, we sought to optimize the temperature and pH in order to increase the rate of hydrolysis with respect to the mutarotation and oxygen-exchange rates.[34] The strong pH dependencies of the mutarotation and oxygen-exchange reactions required the use of a pH greater than 2.[21b,22] The stability of sucrose to hydrolysis at these pH values led to our use of an elevated reaction temperature. Reaction media containing sodium chloride and ^2H_2O (rather than H_2O) increase the rate of hydrolysis with respect to mutarotation;[21,35] sodium chloride also lowers the oxygen-exchange rate.[17b,25] We also found that in the presence of sodium chloride the ^{13}C NMR resonance of the sucrose (glucosyl C-1) carbon is shifted with respect to the α-glucose anomeric carbon, making resolution of these otherwise overlapping peaks[36] possible. Relatively high concentrations of sucrose were used to overcome the inherent lack of sensitivity of (natural-abundance) ^{13}C NMR; as pointed out above, the sucrose concentration did not appear to alter the nature of the reaction. Furthermore, a recent investigation has shown that the solution conformation of sucrose is independent of temperature and concentration over a wide range of these parameters, including those used in this study.[37]

The results presented here are most simply interpreted in terms of fructosyl–(bridge) oxygen bond fission in the rate-determining step of the acid-catalyzed hydrolysis of sucrose. By this mechanism, the initial products of the hydrolysis are α-D-glucose and a fructosyl carboxonium ion. This mechanism is consistent with evidence that a five-membered ring tertiary carboxonium ion is more stable than a six-membered ring secondary carboxonium ion.[11] Our results do not rule out certain alternative mechanisms such as one involving initial cleavage at the fructosyl–(ring) oxygen.[38] Elucidation of this and other more subtle mechanistic details, such as the site(s) of protonation essential for the bond-cleavage reaction, will require alternative experimental techniques.

Soon after the development of the polarimeter in 1840, the hydrolysis of sucrose was the subject of the first kinetic study of a homogeneous chemical reaction.[3,39] That the hydrolysis of sucrose came to be known as the "inversion" of sucrose (due to the positive optical rotation of the substrate and negative optical rotation of the products) is a testimony to the dominance of this technique in investigations of this reaction. However, polarimetry lacked the ability to resolve adequately the many species present during the hydrolysis. By contrast, ^{13}C NMR can resolve these species and, in fact, even the isotopomers that are present when [^{18}O]water is used. The ^{18}O isotope shift in ^{13}C NMR is clearly a powerful tool for elucidating the mechanisms of chemical reactions involving the formation or cleavage of carbon–oxygen bonds.

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Appendix

The solution to the nonhomogeneous series of the differential equations (1) is given in eq 2 for $i = 1$–4.

$$y_i(t) = c_{i1}e^{-kt} + c_{i2}e^{-(k_1+k_2)t} + c_{i3}e^{-k_3t} + c_{i4}e^{-(k_1+k_2+k_3)t} + c_{i5} \quad (2)$$

The coefficients for (2) are the following:

$$c_{i1} = \frac{S_0}{k'(k'-k_m)}\begin{bmatrix} k(k_2-k') \\ kk_1 \\ (k_3(k-k_2)(k'-k_2) + k_1k_2k_3)/(k-k_m) \\ -k_1k_3(k'+k-k_m)/(k-k_m) \end{bmatrix}$$

$$c_{i2} = \frac{S_0kk_1}{k_m(k-k_m)}\begin{bmatrix} 0 \\ 0 \\ 1 \\ -1 \end{bmatrix} \qquad c_{i4} = \frac{S_0kk_1}{k_m(k'-k_m)}\begin{bmatrix} 1 \\ -1 \\ -1 \\ 1 \end{bmatrix}$$

$$c_{i3} = \frac{S_0k}{k'k_m}\begin{bmatrix} k_2 \\ k_1 \\ -k_2 \\ -k_1 \end{bmatrix} \qquad c_{i5} = \frac{S_0}{k_m}\begin{bmatrix} 0 \\ 0 \\ k_2 \\ k_1 \end{bmatrix}$$

where the mutarotation constant $k_m = k_1 + k_2$ and the parameter $k' = k - k_3$ have been introduced to simplify the expressions.

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