Acid-Catalyzed Inversion of Sucrose in the Amorphous State at Very Low Levels of Residual Water

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Purpose. Factors affecting the solid-state acid-catalyzed inversion of amorphous sucrose to glucose and fructose in the presence of colyophilized citric acid, with less than 0.1% w/w residual water, have been studied.

Methods. Samples of citric acid and sucrose were lyophilized at a weight ratio of 1:10 citric acid:sucrose from solutions with initial pH values of 1.87, 2.03, and 2.43, as well as at a weight ratio of 1:5, at an initial pH of 1.87. Glass transition temperatures, Tg, were measured by DSC and the presence of any possible residual water was monitored by Karl Fischer Titrimetry. The inversion of sucrose was measured by polarimetric analysis after reconstitution of solid samples stored at 50°C under P_2O_5.

Results. Samples of 1:10 citric acid:sucrose at an initial pH of 1.87, 2.03, and 2.43 exhibited the same Tg. The initial rate of reactivity was affected at a 1:10 ratio by the solution pH before lyophilization in the order: 1.87 > 2.03 > 2.43 and by citric acid concentration at pH 1.87 in the order 1:5 > 1:10.

Conclusions. Sucrose, colyophilized with an acid such as citric acid, undergoes significant acid-catalyzed inversion at 50°C despite the very low levels of residual water, i.e., <0.1% w/w. At the same ratio of citric acid to sucrose (1:10), and hence the same Tg, the rate of reaction correlates with the initial solution pH indicating that the degree of ionization of citric acid in solution is most likely retained in the solid state. That protonation of sucrose by citric acid is important is shown by the direct relationship between maximum extent of reaction and citric acid composition. It is concluded that colyophilization of acidic substances with sucrose, even in the absence of residual water, can produce reducing sugars capable of further reaction with other formulation ingredients susceptible to reaction with reducing sugars.

KEY WORDS: amorphous; sucrose; acid-catalyzed; residual water; solid-state degradation.

INTRODUCTION

During the course of carrying out experiments with relatively dry (<0.1% w/w water) freeze-dried amorphous mixtures of an organic drug molecule, spirapril HCl, (1,4diatha-7-azaspirol[4,4]nonane-8-carboxylic acid, 7-[2[[1-ethoxycarbonyl)-3-phenylpropylamino]-1-oxopropyl]-monohydrochloride) and either sucrose or trehalose in a 1:10 weight ratio of drug to sugar, certain interesting observations were made after storing these samples at 50°C for up to about one month. In the case of the trehalose system, no apparent physical or chemical changes appeared to occur; however, with the sucrose system, the original white cake became progressively colored and eventually collapsed to a very small volume. Based on previously reported studies with acidic solutions of sucrose (1), and particularly with freeze-dried sucrose at acidic pH conditions and in the presence of residual water (2–4), it was concluded that sucrose most likely had undergone acid-catalyzed inversion to glucose and fructose, both reducing sugars. The coloration during this process was believed to be the result of additional reactivity between the drug and reducing sugars that produced non-enzymatic browning (5,6). The acidity needed to catalyze this reaction most likely came from the acidic nature of the drug.

That such a reaction can occur in the presence of acidic species for amorphous sucrose at low levels of water, as most likely observed above, was shown by Karel & Labuza with lyophilized formulations of sucrose, citric acid, and microcrystalline cellulose stored at 55°C with a water content as low as 0.22% w/w, in the weight ratio of sucrose:microcrystalline cellulose:citric acid of 30:2:1 (6). In the absence of citric acid negligible reaction took place at 55°C over the same time period. It was concluded that at amounts of residual water well below the so-called "BET-monolayer" level, as estimated from water vapor sorption isotherms (7), water still played an important role in the reaction mechanism. How this might occur at such low levels, and how citric acid might be mechanistically involved was not considered further.

Water in such an amorphous system can be expected to have at least 3 important functions in this reaction. It can act as a reactant, as a plasticizer to increase molecular mobility and as a medium supporting acid-base reactions involved in the overall chemical mechanism (8). Given the very low levels of water in our system, <0.1%, relative to sucrose, one might not have expected that such an extensive chemical change could occur due to reaction with water over a relatively short time period, i.e., 2–4 weeks at around 50°C. Given that amorphous sucrose has a glass transition temperature of about 74°C (9), even in the presence of 0.1–0.2% water, storage at 50°C would be at a temperature well below the glass transition temperature, Tg, (spirapril HCl has a Tg of about 85°C and would be expected to slightly raise the Tg in a mixture with sucrose (10)). Thus, we would not expect a significant role of water at such low levels as a plasticizer. This suggests that "pH" effects at such low levels of water in these systems are most likely the most important factors. This suggestion is supported by the work of Richards who reported studies on the thermal degradation of sucrose in the melt over the temperature range of 120°C to 180°C for a period of about 400 minutes in the complete absence of water (11). It was concluded that traces of initial degradation reactions produced acidic substances, such as acetic, formic, and levilinic acids, which in turn protonated sucrose and facilitated degradation. Thus, it seems reasonable that in the present case the role of any acid present might be enough to explain the results of studies carried out in the range of 50°–55°C in the absence of any significant amounts of water, if there was sufficient molecular mobility of sucrose and acid at the temperature of the reaction.

To examine the tendencies of sucrose to undergo acid-catalyzed inversion at 50°C under the conditions of very low water content and to examine the role of any acid present,

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we have replaced spirapril with citric acid. We have prepared samples at a citric acid:sucrose weight ratio of 1:10 by lyophilization with different solution concentrations of citric acid and sucrose so as to bring the initial pH to values of 1.87, 2.05, and 2.43 while keeping the composition of citric acid and sucrose at the 1:10 level. This was of interest since it had recently been suggested that lyophilized proteins exhibited "pH memory" of the aqueous solution from which they were lyophilized and its initial pH conditions (12). A study was also carried out using a 1:5 molar weight ratio of citric acid to sucrose where the solution was adjusted to pH 1.87.

MATERIALS AND METHODS

Sucrose (Mallinckrodt, AR grade) and citric acid monohydrate (Mallinkrodt, USP grade) were used as received. Water used for lyophilization and polarimetric analysis was deionized using a Barnstead water purification system. Two solid citric acid:sucrose weight ratios of 1:10 and 1:5 were prepared by lyophilization of aqueous solutions of citric acid and sucrose. One group of solutions containing 31.6% and 40.1% w/w of total solids, representing 1:10 and 1:5 ratios, respectively, were adjusted to pH 1.87. In addition, two other solutions at a 1:10 ratio were prepared with 19.4% and 5.5% total solids, to give initial solution pH values of 2.05 and 2.43, respectively. Freeze-drying was performed in a Dura-Stop DC freeze-dryer (FTS Systems, Stone Ridge, NY). Solutions were transferred into petri dishes and frozen with liquid nitrogen immediately after the solutions were made to minimize sucrose inversion in solution. The frozen solutions were placed in the freeze-drier at −40°C, immediately after which vacuum was applied. At the residual pressure of 50–70 mT, the temperature was maintained at −40°C for 4 days and then increased to 25°C at about 5°/day for at least 5 more days. Analysis of samples for any chemical conversion of sucrose during freeze drying indicated no significant degradation and there was no collapse of the freeze-dried cake. The lyophilized material was gently ground with a mortar and pestle, placed into 2 ml vials, and dried over phosphorus pentoxide at room temperature in a desiccator under vacuum for at least 6 days. The material was then placed in a desiccator over phosphorus pentoxide under vacuum for 1 day at 50°C to completely dry the samples. All operations with freeze-dried materials were performed in a glove box under nitrogen flow. Residual water content for all solid samples was determined by the Karl Fischer method using an Aquastar C2000 coulometric titrator (EM Science, Cherry Hill, NJ). It was shown that all samples treated in the manner described above contained no more than 0.1% water, the lower limit of sensitivity of this technique.

Powder X-ray diffraction patterns were obtained with a PadV, Scintag Powder X-ray Diffractometer (Scintag, Inc., Santa Clara, CA) controlled by a computer (Model B10610, Tetronix, Inc., Wilsonville, OR). The radiation used was generated by a copper Kα filter with a wavelength of 1.5418 Å at 45 kV and 40 mA. Samples were scanned from 5° to 40° 2θ at a scanning rate of 5° per minute. In all cases initial and reacted samples exhibited no diffraction peaks and had the halo characteristic of a completely amorphous phase.

Differential Scanning Calorimetry (DSC) measurements were carried out on all amorphous samples to measure the glass transition temperature, Tg, using a Seiko SSC/5200 instrument (Seiko Instruments, Horsham, PA). The instrument was calibrated for temperature using the melting point of high purity indium, tin, and gallium. Samples of 3–13 mg were placed into hermetically sealed pans under dry-box conditions of low relative humidity and a nitrogen atmosphere. Typically, samples were cooled to −20°C and heated at a rate of 10°C/min to 95°C, in order to determine the onset Tg, the point at which a change in heat capacity first occurs. This will be referred to as the first scan. Generally, this heating and cooling cycle was repeated once more to give what will be referred to later as a second scan.

Kinetic studies of chemical reactivity were carried out in open 2 ml vials in desiccators over phosphorus pentoxide under vacuum at 50° ± 1°C using a laboratory oven. Vials were removed periodically and subjected to DSC and XRD analysis, as described above, and to polarimetric analysis in solution as described below. The possible effects of any phosphoric acid formed during storage in causing chemical changes of sucrose were addressed by periodically changing the P_2O_5 during long-term storage. Also we found no decomposition of pure sucrose over the entire storage period and no solution pH change before and after freeze drying, thus supporting the conclusion that no such effects occurred.

The inversion of sucrose to glucose and fructose was followed with time using a Perkin-Elmer model 121 polarimeter. It had been shown previously that results using polarimetry to follow this reaction in solution and with solid materials containing residual water were consistent with those obtained using other methods (13). Weighed amounts of sample were dissolved in water to obtain about a 3% w/w solution. The extent of sucrose inversion, x, was calculated using the following equation:

$$x = \frac{\alpha_s - \alpha}{\alpha_s - \alpha_i} \tag{1}$$

where α_s is the specific rotation of the 100% sucrose sample, equal to 66.4 (14); α is the specific rotation of the sample under consideration, and α_i is the specific rotation of the pure invert sugar, equal to −19.7 (15). Specific rotation of the sample was calculated as

$$\alpha = \frac{100\ \alpha_c}{LC_S} \tag{2}$$

where α_c is the instrument reading, C_S is the weight percent of solute in the solution, and L is the length of the cuvette.

In general the optical rotation of the solution samples, representing various extents of chemical reaction, decreased with time and asymptotically approached a constant value after about 2–3 hours at room temperature. These changes are due to the mutorotation of the fructose and glucose produced as the result of sucrose inversion (13). The extent of sucrose inversion, therefore, was calculated using solutions maintained for two or more hours after dissolution of the solid sample in the deionized water, to correct for the mutorotation lag. The relative uncertainty in the determination of the extent of chemical changes is estimated to be within one percent.

RESULTS

Table I lists the various samples used in this study, including the citric acid:sucrose weight ratio, initial solution pH, the

Table I. Sample Composition and Tg in Colyophilized Sucrose:Citric Acid Mixtures

Citric acid: sucrose wt ratio	Initial pH	Tg,[a] 1st scan	Tg, 2nd scan	Tg calculated from (Eq. 3) using second scan data for sucrose Tg (°C)
0:1	5.87	80	72	—
1:10	1.87	68	57	66
1:10	2.05	68	57	66
1:10	2.43	69	58	66
1:5	1.87	69	52	61

[a] Annealed at 50°C for 24 hours prior to measurement.

glass transition temperature measured from the first DSC scans after annealing samples at 50°C for one day and a Tg obtained from a second scan. Usually, a second scan is used to report Tg since the first scan eliminates the previous history of the sample and allows uniformity in reported values. However, in this study, chemical reactivity occurs in samples having a particular thermal history, i.e., in samples annealed at 50°C, which is below Tg. Hence, it is believed to be important to report values of Tg for both annealed and unannealed samples. The Tg for pure amorphous sucrose, treated in this manner was in good agreement with previous results (9). The Tg of pure citric acid has been reported to be 11°C (16). As can be seen in Table 1, the two citric acid:sucrose mixtures had lower values of Tg than sucrose, indicating the plasticizing effects of citric acid. Also listed in Table 1 are estimates of Tg for these mixtures assuming ideal mixing behavior, as reflected in the Gordon Taylor equation (17) where

$$Tg = \frac{w_1 Tg_1 + K w_2 Tg_2}{w_1 + K w_2} \quad (3)$$

and

$$K \approx \frac{Tg_1 \rho_1}{Tg_2 \rho_2} \quad (4)$$

where w_1 and w_2 are weight fractions and ρ_1 and ρ_2 are densities of each component. The density of amorphous sucrose is 1.43 gcm^{-3} (9) and that of amorphous citric acid is 1.61 gcm^{-3} (16). It can be seen that the experimental values of Tg are lower than those predicted by equation 3, indicative of the non-ideal mixing of the two components in the molecular dispersion formed by lyophilization (18).

The kinetic curves for the loss of sucrose with time, taken as an average of at least two independent samples, are shown in Fig. 1. Here, it can be seen that significant degradation occurs at 50°C, for the 1:10 citric acid:sucrose samples at 3 initial solution pH values; the rates increase as the initial solution pH decreases. Also included in Fig. 1 are the results obtained for amorphous sucrose, prepared in the same manner, but without citric acid present. In this case no chemical change occurred over comparable time periods. In Fig. 1 we also present a comparison of the changes occurring with the 1:10 and 1:5 citric acid-sucrose samples, lyophilized from a pH 1.87 solution. Here it can be seen that the rate of sucrose reactivity is greater the higher the citric acid concentration. It is also apparent from Fig. 1 that the maximum extent of reaction was greater in the mixture with the higher citric acid level.

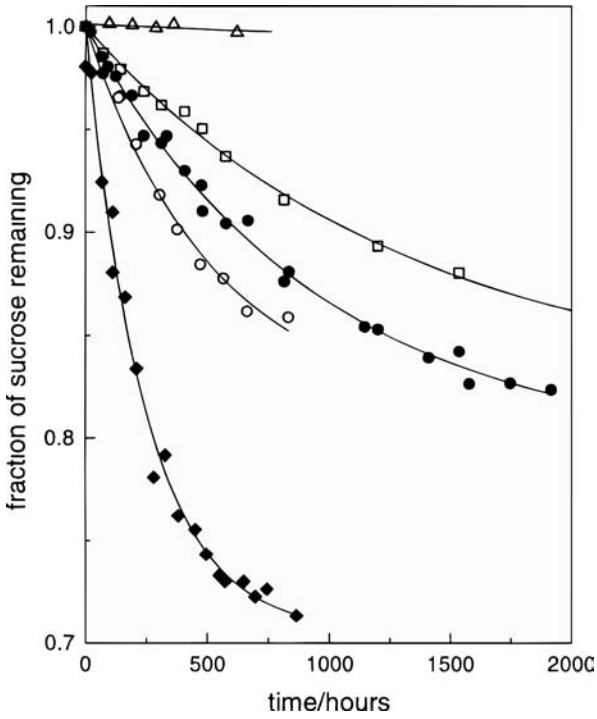

Fig. 1. Kinetic curves for sucrose:citric acid freeze-dried mixtures. Results for pure sucrose (Δ) are also given for comparison. □: sucrose:citric acid = 10:1, freeze-dried from solution with pH 2.4; ●: sucrose:citric acid = 10:1, freeze-dried from solution with pH 2.05; ○: sucrose:citric acid = 10:1, freeze-dried from solution with pH 1.87; ◆: sucrose:citric acid = 5:1; freeze-dried from solution with pH 1.87. Lines present fitting of the experimental data using Eq. 5.

DISCUSSION

The results of these experiments would indicate that citric acid has a major effect on this reaction in the absence of a significant amount of water. The influence of the acid on this reaction is consistent with conclusions made from studies in solution and the melt (1,11,19) that acidic species are directly involved in the inversion of sucrose. The prevailing view, as seen in Scheme 1, therefore, would suggest that in solution a rapid pre-equilibration protonation (step 1) followed by a unimolecular rate-determining splitting of the glycosidic bond (step 2) and conversion to glucose and fructose (step 3) occurs:

Scheme 1

1) $H^+ + S \rightleftharpoons SH^+$

2) $SH^+ \rightarrow GH^+ + F$

or

$SH^+ \rightarrow FH^+ + G$

3) $GH^+ + H_2O \rightarrow G + H^+$

or

$FH^+ + H_2O \rightarrow F + H^+$

where S, G, and F represent sucrose, glucose, and fructose, respectively.

The species formed in step 2 can be either glucose or fructose depending on the site of bond cleavage. Glycosyl-oxygen cleavage will produce fructose and glucose ion, whereas fructosyl-oxygen cleavage will produce glucose and fructose ion. Both processes have been shown to occur in solution (20). According to this scheme, therefore, water does not participate as a direct reactant in sucrose cleavage, but it does participate in the later conversion of the carbonium ions into neutral monosaccharides.

To compare the rates of reactivity in samples with different sets of conditions, we have carried out the following kinetic analysis. In solution, sucrose inversion is generally described by a first-order equation where the experimental rate constant is equal to a constant k times $[H^+]$ (1). It is usually assumed that in solution $[H^+]$ is constant during the reaction, but this undoubtably is not the case in the present study where citric acid is most likely the major and limited source of protons. In Scheme 1, we see the importance of citric acid in producing protonated sucrose and the importance of water in facilitating the final conversion of monosaccharide carbonium ions. Consequently, the amount of citric acid present in the sample initially in the presence of negligible amounts of water should limit the extent to which this reaction can proceed and, indeed, from the curves in Fig. 1 it can be seen that the extent of reaction over the entire time frame is greater the higher the level of citric acid at the same initial solution pH before freeze drying.

The effect of citric acid as a limiting factor for the maximum extent of reaction can be expressed in a more quantitative basis by considering the overall reaction to consist of several parallel and consecutive elementary reactions. To simplify the analysis, we assume that unimolecular cleavage of sucrose is the rate-determining step as has been known to be true in solution (19). Furthermore, we assume that sucrose cleavage is an irreversible reaction and that the maximum extent of conversion is determined by the extent of sucrose protonation possible. In other words, we assume that there are two groups of sucrose molecules present, the protonated species capable of reacting by first-order kinetics, and a nonreactive nonprotonated species. This allows us to express the extent of reaction, α at any time, t, as

$$\alpha(t) = X_m \exp(-kt) + (1 - X_m) \quad (5)$$

where X_m is the weight fraction of protonated sucrose (SH^+) representing the maximum extent of reaction, and k is a rate constant characterizing the rate of reactivity. Table II presents the values of k and X_m estimated for data presented in Fig. 1. Fits to equation 5 were obtained with k and X_m as adjustable parameters using a Microcal Origin software package.

Table II. Rate Constants (k) and Maximum Extents of Reaction (Xm) for Colyophilized Citric Acid:Sucrose Mixtures Obtained by Fitting of the Experimental Data to Eq. 5 (see text)

Citric Acid:Sucrose	Initial pH	$k \times 10^3$ hr^{-1}	Xm
0:1	5.87	0	0
1:10	2.43	0.76 ± 0.09	0.176 ± 0.014
1:10	2.05	1.05 ± 0.06	0.206 ± 0.006
1:10	1.87	1.91 ± 0.27	0.185 ± 0.015
1:5	1.87	37.8 ± 5.2	0.267 ± 0.014

It should be pointed out that this kinetic analysis is oversimplified, since while it allowed us to explain the greater extent of reaction in the mixture with a higher citric acid (and higher proton) level, and the similar values of Xm for all 1:10 samples, it does not explain why the initial rate of reactivity was higher in the citric acid:sucrose 1:5 mixture. Consequently, our results can only be used to compare rates and maximum extents of reactivity and we must be cautious about speculations concerning exact mechanisms. From this analysis, we can conclude that the maximum extent of the reaction is directly related to the amount of citric acid present, presumably a limitation on the amount of protonated sucrose that can form. The generally enhanced rate of reactivity likewise at the same "pH," but with a different ratio of citric acid present, again, most likely reflects the role of citric acid in protonating sucrose.

From Table I we note that the citric acid-sucrose samples all have about the same first-scan Tg values and, therefore, on this basis alone they would be expected to have similar rates of solid-state reactivity if molecular mobility was the predominant factor. However, from Fig. 1 and Table II it is apparent that initial rates as reflected in the constant k are quite different, which we believe illustrates the importance of medium effects on chemical reactions in the amorphous state, in general.

CONCLUSIONS

This study has shown the significant sensitivity of amorphous sucrose to degradation in the presence of a molecularly dispersed acidic solid at temperatures as low as 50°C and in the presence of negligible amounts of residual water. It is further shown that the initial rate of reactivity depends on the pH of the solution used to prepare the lyophilized sample and that the maximum extent of reaction is limited by the amount of acid present and, most likely, its role in the formation of an important intermediate, the protonated sucrose molecule. Consequently, even in the relatively dry state, lyophilized samples containing sucrose and some acidic ingredients are susceptible to chemical degradation under conditions of storage that could easily be encountered during stability assessment and the shelf-life of the product.

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REFERENCES

1. P. M. Leninger and M. Kilpatrick. The inversion of sucrose. *J. Am. Chem. Soc.* **60**:2891–2899 (1938).
2. T. Schoebel, S. R. Tannenbaum, and T. P. Labuza. Reaction at limited water concentration 1. Sucrose hydrolysis. *J. Food Sci.* **34**:324–329 (1969).
3. M. P. W. M. te Booy, R. A. de Ruiter, and A. L. J. de Meere. Evaluation of physical stability of freeze-dried sucrose-containing formulations by differential scanning calorimetry. *Pharm. Res.* **9**:109–114 (1992).

4. M. del Pilar Buera, J. Chirife, and M. Karel. A study of acid-catalyzed sucrose hydrolysis in an amorphous polymeric matrix at reduced water contents. *Food Res. Intern.* **28**:359–365 (1995).
5. L. N. Bell. Kinetics of non-enzymatic browning in amorphous solid systems: distinguishing the effects of water activity and glass transition. *Food Res. Intern.* **28**:591–597 (1996).
6. M. Karel and T. P. Labuza. Nonenzymatic browning in model systems containing sucrose. *J. Agr. Food Chem.* **16**:717–719 (1968).
7. R. B. Anderson. Modifications of the Brunauer, Emmett and Teller equation. *J. Am. Chem. Soc.* **68**:686–691 (1946).
8. E. Y. Shalaev and G. Zografi. How does residual water affect the solid-state degradation of drugs in the amorphous state? *J. Pharm. Sci.* **85**:1137–1141 (1996).
9. A. Saleki-Gerhardt and G. Zografi. Nonisothermal and isothermal crystallization of sucrose from the amorphous state. *Pharm. Res.* **11**:1166–1173 (1994).
10. W. Xu. Investigation of Solid-State Stability of Selected Bioactive Compounds. Ph.D. Thesis, Purdue University, 1997.
11. G. N. Richards. Initial steps in the thermal degradation of sucrose. *Int. Sugar Jnl.* **88**:145–148 (1986).
12. H. R. Constantino, K. Griebenow, R. Langer, and A. M. Klibanov. On the pH memory of lyophilized compounds containing protein functional groups. *Biotech. Bioeng.* **53**:345–348 (1997).
13. S. Buchanan, D. G. Kubler, C. Meigs, M. Owens, and A. Tallman. Energy of activation and temperature for the hydrolysis of sucrose. *Int. J. Chem. Kinetics.* **15**:1229–1234 (1983).
14. W. C. Vosburgh. The optical rotation of mixtures of sucrose, glucose and fructose. *J. Am. Chem. Soc.* **43**:219–232 (1921).
15. CRC Handbook of Chemistry and Physics. 66th edition. R. C. Weast, M. J. Astle, and W. H. Beyer (Eds.), p. E-410.
16. Q. Lu and G. Zografi. Properties of citric acid at the glass transition. *J. Pharm. Sci.* **82**:657–659 (1997).
17. M. Gordon and J. S. Taylor. Ideal copolymers and the second-order transitions of synthetic rubbers, I. non-crystalline copolymers. *J. Appl. Chem.* **2**:493–500 (1952).
18. S. L. Shamblin, L. S. Taylor, and G. Zografi. Mixing tendencies of co-lyophilized sucrose-additive systems. *J. Pharm. Sci.* **87**:694–701 (1998).
19. J. H. Pazur. Oligosaccharides. In W. Pigmann, D. Horton, and A. Herp (eds.), *The Carbohydrates. Chemistry and Biochemistry*, Academic Press, New York, 1970, pp. 96–97.
20. T. L. Mega and R. V. Etten. The ^{18}O isotope shift in ^{13}C nuclear magnetic resonance spectroscopy. 12. Position of bond cleavage in the acid-catalyzed hydrolysis of sucrose. *J. Am. Chem. Soc.* **110**:6372–6376 (1988).