Abstract: 6,6'-Dithiosucrose (sucrose dithiol, Suc(SH)₂) is a weakly reducing species. The equilibrium constant for reduction of mercaptoethanol disulfide, ME²⁺, by sucrose dithiol in aqueous solution is \( K_{eq} = 0.3 \text{ M} = \left( \frac{[\text{MeS}]}{[\text{MeS}]} \right) \left( \frac{[\text{SucSH}]}{[\text{ME}]} \right) \), where Suc(SH)₂ (6,6'-dithiosucrose cyclic disulfide, sucrose disulfide) is the cyclic disulfide formed from the oxidation of sucrose dithiol. Measurements of values of \( T \) and \( J \) using \(^1H\) NMR spectroscopy indicate that sucrose and sucrose dithiol adopt indistinguishable conformations in water. The conformations of sucrose dithiol and sucrose disulfide are similar but distinguishable.

Molecular mechanics calculations indicate that two of the possible structures of sucrose disulfide have relatively low energies. Comparison of coupling constants calculated for these structures with experimental coupling constants from \(^1H\) NMR spectra indicated that one of the two structures was more probable; this more probable structure had the conformation more similar to that of sucrose. The reduction potential of the Suc(SH)₂/SucS₂ (an 11-membered ring) couple is similar to that of an n-alkane-1, n-dithiol/cyclic disulfide (n = 5 or 6) couple; the value of EC (effective concentration = \( K_{eq} = 0.3 \text{ M} \)) characterizing oxidation of the two thiols of Suc(SH)₂ to a disulfide is similar to that for oxidation of the two thiols of hexane-1, 6-dithiol (\( K_{eq} = 0.2 \text{ M} \)) and pentane-1, 5-dithiol (\( K_{eq} = 3.6 \text{ M} \)). The similarity of these values of EC suggests that SucS₂ is a relatively strain-free structure and reflects the proximity of the two thiol groups in this carbohydrate.

Introduction

This paper explores the usefulness of the redox reaction linking thiols and disulfides (eq 1) in defining the conformation of the representative disaccharide sucrose (\( \alpha \text{Glc}(1\rightarrow2)\beta \text{Fru} \)). The thiol-disulfide interchange reaction has been developed by Creighton and by Kim as a probe of conformations in biochemical systems, particularly in studies of oligopeptides containing two cysteine moieties. The assumption underlying these studies is that the redox potential of a dithiol reflects the geometry of the SH groups; that is, the tendency for the dithiol to be oxidized to the corresponding disulfide is higher if the favored conformation of the thiol reactant resembles that of the disulfide product. Measurement of the change in free energy for oxidation of a dithiol to a disulfide is normally accomplished using thiol-disulfide interchange. Direct electrochemical measurements of redox potentials for thiol-disulfide couples are not reliable because the surfaces of most electrodes are reactive to organosulfur compounds and the redox reactions are typically not thermodynamically reversible. The most straightforward experimental measurements of equilibrium constants for strongly reducing thiols (typically, dithiols capable of forming stable cyclic disulfides, eqs 2 and 3) are based on equilibration of these species with a stable cyclic disulfide of known structure and redox potential (e.g., reduced (DTT) and oxidized (DTT⁺) diethithreitol, lipoic acid, and similar materials (eq 4)). This method is, however, difficult to apply to weakly reducing dithiols, and the equilibrium for these species is often measured with respect to a more readily reduced noncyclic disulfide such as oxidized glutathione (GSSG), oxidized mercaptoethanol (ME²⁺), or cysteine (eqs 5 and 6). These systems

\[
\text{HSRSH} + \text{SR'S} \rightleftharpoons \text{SRS} + \text{HSR'SH} \quad (2)
\]

\[
K = \frac{[\text{SRS}][\text{HSR'SH}]}{[\text{HSRSH}][\text{SR'S}]} \quad (3)
\]

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\[
\text{HSRSH} + (\text{HOCH}_2\text{CH}_2\text{SH})_2 \rightleftharpoons \text{ME}^{2_-} \quad (4)
\]

\[
\text{SRS} + \text{HOCH}_2\text{CH}_2\text{SH} \rightleftharpoons \text{ME}^{2_-} \quad (5)
\]

\[
K_{ME} = \frac{[\text{SRS}][\text{ME}^{2_-}]}{[\text{ME}][\text{MH}]} = \text{EC} \quad (6)
\]

based on noncyclic disulfides can be applied to weakly reducing dithiols, since the concentrations of the equilibrating species can be adjusted to values convenient for the measurements of the relevant concentrations. By convention, this equilibrium

\[
\text{HSRSH} + \text{SR'S} \rightleftharpoons \text{SRS} + \text{HSR'SH} \quad (2)
\]

\[
K = \frac{[\text{SRS}][\text{HSR'SH}]}{[\text{HSRSH}][\text{SR'S}]} \quad (3)
\]

\[
\epsilon = \epsilon_{DTT} - 0.029 58 \log \frac{[\text{DTT}][\text{cyclic disulfide}]}{[\text{DTT}][\text{dithiol}]} \quad (4)
\]

\[
\text{HSRSH} + (\text{HOCH}_2\text{CH}_2\text{SH})_2 \rightleftharpoons \text{ME}^{2_-} \quad (4)
\]

\[
\text{SRS} + \text{HOCH}_2\text{CH}_2\text{SH} \rightleftharpoons \text{ME}^{2_-} \quad (5)
\]

\[
K_{ME} = \frac{[\text{SRS}][\text{ME}^{2_-}]}{[\text{ME}][\text{MH}]} = \text{EC} \quad (6)
\]
constant (relative to a noncyclic disulfide) is often given the name "effective concentration" (EC, eq 6) because it has units of concentration. The EC is often interpreted as the concentration of monothiol, RSH, required in an intermolecular reaction (eq 7)

\[
2\text{RSH} + \text{R'SSR'} \rightleftharpoons \text{RSSR} + 2\text{R'SH}
\]

to give the same conversion to product as is achieved by HSRSH when the two reactions are conducted under similar conditions. The energy of a CSSC unit depends strongly on the CSSC dihedral angle and constraints on this angle may be very different in an intramolecular reaction and an intermolecular "model" for it, interpretation of EC (especially low values of EC) in terms of the proximity of the thiol groups must be guarded. In a hypothetical situation in which the distance of S' from S in Figure 1A is the same as that of S' from S in B (that is, the "concentration" is the same) but the CSSC dihedral angle is 90° in A and 0° in B, the equilibrium will still lie strongly in favor of A.

In principle, thiol-disulfide interchange is a particularly attractive reaction with which to measure values of EC (or equilibrium constant) and to draw approximate inferences concerning proximity and conformation. The equilibration of thiols with disulfides can be accomplished at room temperature in water at neutral or alkaline values of pH or in polar organic solvents. The reaction occurs in very high yield, and its mechanism is simple and well understood. Kim correctly emphasized, when using this reaction to study conformations in polypeptides, that it is sufficient (and is experimentally a great simplification) to measure only the concentrations of the reacting species used to define the value of EC; other species (polymers, mixed disulfides) need not be considered. The system is thus analytically tractable.

The physical organic chemistry of thiol-disulfide interchange is well established. Interactions between thiols and disulfides involves initial deprotonation of the thiol to thiolate, nucleophilic attack of the thiolate anion on the disulfide group along the S-S axis, and protonation of the new thiolate anion generated in this process. For monothiols, the rates and equilibrium constants for many thiol-disulfide interchange reactions follow Brønsted relationships. Reference values of EC (either in that form or as values of equilibrium constants or of \( K \)) are available for many compounds, especially for \( \alpha,\omega \)-dithiols that form cyclic monomeric disulfides upon oxidation. A comparison of values of EC from model compounds with the value of EC for a new cyclic monomeric disulfide provides a measure of the stability of the latter. The further interpretation of this number in terms of conformation is one subject of this study.

This paper reports the first test of the value of measurements of EC based on thiol-disulfide interchange to examine the conformations of disaccharides in solution. As a test case, we determined the equilibrium constant for sucrose having hydroxyl groups on the 6 and 6′ carbon atoms replaced by thiols (eq 8).
Scheme 1. Synthesis of Sucrose Disulfide 4

1) PPh₃, CBr₄, Pyr
2) Ac₂O, pyr 73%

1) Sodium Methoxide, Methanol
2) Dowex 50 H⁺ form

1) Na₂CO₃, air oxidation, 24 h, H₂O, r.t.
2) AcOH
3) AqO, Pyr

Lees and Whitesides

Figure 2. Neutron diffraction structure of sucrose.²⁷ O-6 and O-6' are 4.0 Å apart. Oxygen and hydrogens are labeled according to the nearest carbon.

Results and Discussion

Synthesis. Scheme 1 outlines an adaptation of the method of Hough for the synthesis of 6,6'-dithiosucrose. We oxidized the dithiol to the disulfide by stirring the dithiol at low concentration in a solution saturated with air. We quenched the oxidation previously thought and suggested that the hydrogen bond between the hydroxyl group at C-2 and the C-1' hydroxyl is not important in determining the conformation and that this hydrogen bond is not persistent in solution.²⁰

References

reaction with acetic acid, acetylated the disulfide with acetic anhydride, and purified it by column chromatography on silica gel. Deacetylation in base afforded the free sucrose disulfide. In addition, two dimeric cyclic disulfides (head-to-head and head-to-tail) were isolated as minor products of this reaction.

**Characterization. Monomer.** We assigned the resonances in the $^1$H NMR spectrum of the peracetylated cyclic monomer of sucrose disulfide (acetylated sucrose disulfide) using a 2D HOMOCOSY experiment. At room temperature in CDCl$_3$, the $^1$H NMR spectrum of the acetylated sucrose disulfide showed broad lines due to conformational interchange (Figure 3). At 273 K the $^1$H NMR spectrum of this molecule indicated the presence of two conformations in a ratio of ca. 10:1. The $^1$H NMR peaks due to the two conformations coalesced above 273 K. Below 273 K, the peaks in the $^1$H NMR spectrum of the two conformers broadened further due to lower energy conformational exchange, possibly "ring flipping". The peaks in the $^{13}$C NMR spectrum were partially assigned using a $^1$H/$^{13}$C 2D HETEROCOSY experiment.

The resonances in the $^1$H NMR spectrum of sucrose disulfide (formed by deacetylating the cyclic monomer of sucrose disulfide hexaacetate) were also assigned using a 2D HOMOCOSY experiment (Figure 4). At room temperature in D$_2$O, the $^1$H NMR spectrum of sucrose disulfide showed broad lines due to conformational interchange. At 353 K, the peaks of the sucrose disulfide were sharp and represented a single time-averaged conformation (Figure 5). The freezing point of water prevented spectral observation of the free cyclic monomer at temperatures below 273 K.

**Dimer.** Several observations support the assignment of a dimeric structure (head-to-head; head-to-tail) to two of the minor products of the oxidation: (1) FAB mass spectrometric analysis of this peracetylated material showed an M + 1 peak consistent with the molecular weight of the dimer; (2) vapor-phase osmometry measurements of the peracetylated material gave an approximate molecular weight (1390 g/mol) consistent with that of a dimer (1248 g/mol); (3) retention times using HPLC and TLC were longer for the free sucrose disulfide dimer than for the free cyclic...
monomer of sucrose disulfide; (4) Ellman's test27 of the per-
acetylated and free dimers indicated that there were no free thiols; and (5) reduction of the free dimers with DTT regenerated sucrose di-
thiol and indicated that formation of dimer was reversible under mild conditions. We did not determine which species was the head-
to-head dimer and which was the head-to-tail dimer. The 1H and 13C NMR spectra of the two dimers showed no confor-
nomational exchange on the NMR time scales at 298 K.

Measurement of Equilibria for Thiol–Disulfide Interchange of Sucrose Dithiol. Preliminary experiments established that sucrose disulfide could be observed by equilibration of sucrose dithiol and the disulfide of β-mercaptoethanol.28 We determined the equilibrium constant \( K_{eq} \) (eq 6, \( R = \text{sucrose} \)) of sucrose dithiol and mercaptoethanol disulfide at several concentrations using \(^1\)H NMR spectroscopy. By integrating the peaks characteristic of the four compounds—the \( \text{H-1} \) peak of the cyclic monomeric sucrose disulfide, the \( \text{H-6b} \) peak of the sucrose dithiol, and the peaks due to the methylene protons adjacent to the sulfur atoms in mercaptoethanol and mercaptoethanol disulfide—we determined the mole compositions of the sucrose dithiol and disulfide isomers (typically HS-sucrose-(S-S-sucrose)_n-SH) and also present; their concentrations depended on the initial concentrations of sucrose dithiol and mercaptoethanol disulfide. Figure 6 shows a representative spectrum of an equilibrium mixture at 25 °C. The measured value of EC was constant over a range of concentrations of sucrose dithiol and mercaptoethanol disulfide from 4 to 40 mM. At concentrations higher than 40 mM, the formation of dimers, polymers, and mixed disulfides involving sucrose dithiol and sucrose disulfide using the crystal structure of sucrose, molecular mechanics, and \(^1\)H NMR spectroscopy. As our initial model we used the crystal structure of sucrose. The crystal structure of sucrose places the C-6 and C-6' oxygens 4.0 Å apart. Rotation of the C-6 hydroxymethyl group by 120° places the two oxygens 2.3 Å apart. This rotamer is the favored conformation for simple glucopyranosides in solution. The replacement of the CH₂OH group at C-6 and C-6' in this new rotamer with CH₂SH implies that the energy difference between the ground state of sucrose disulfide and its transition state for thiol disulfide inter-
change with DTT is almost the same as that for mercapto-
ethanol disulfide. The similar rates of reduction for sucrose disulfide and for a strain-free disulfide (mercaptoethanol disulfide) imply that the disulfide group of sucrose disulfide is also strain-free.

The CSSC Dihedral Angle of Sucrose Disulfide. In an effort to understand the formation of sucrose disulfide in greater detail, we wished to determine the CSSC dihedral angle of sucrose disulfide experimentally. Unfortunately, no presently available technique accurately measures the CSSC dihedral angle in solution.29 UV spectroscopic data are, however, compatible with the hypothesis that the dihedral angle in sucrose disulfide is strainless: the UV spectra of peracetylated sucrose disulfide and of sucrose disulfide show a peak at 250 nm. Absorption at this wavelength is characteristic of unstrained noncyclic disulfides. In the absence of evidence to the contrary (vide infra), we conclude that the CSSC dihedral angle is ca. 90°.30

Conformational Analysis Using Molecular Mechanics. We investigated the lowest energy conformations of sucrose dithiol and sucrose disulfide using the crystal structure of sucrose, molecular mechanics, and \(^1\)H NMR spectroscopy. As our initial model we used the crystal structure of sucrose. The crystal structure of sucrose places the C-6 and C-6' oxygens 4.0 Å apart. Rotation of the C-6 hydroxymethyl group by 120° places the two oxygens 2.3 Å apart. This rotamer is the favored conformation for simple glucopyranosides in solution. The replacement of the CH₂OH group at C-6 and C-6' in this new rotamer with CH₂SH groups places the two sulfur atoms 1.8 Å apart with a CSSC dihedral angle of 130°. Since a typical sulfur–sulfur bond length is 2.05 Å, this conformation of the dithiol or disulfide is unfa-
vorable; thus the sucrose disulfide or sucrose dithiol must deform from this conformation.

Due to the inadequacies in the first model, we investigated the possible conformations of sucrose disulfide by energy minimization of the various C-6 and C-6' rotamers of sucrose disulfide using force-field calculations.31 These calculations produced the two low-energy conformations shown in Figure 7. We hypothesize that interchange between these two conformers could cause the

\[ \text{Table I. EC of Various Cyclic Disulfides} \]

<table>
<thead>
<tr>
<th>Structure</th>
<th>EC (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,6'-sucrose disulfide</td>
<td>0.3</td>
</tr>
<tr>
<td>3.6'</td>
<td>0.2</td>
</tr>
<tr>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>180.0</td>
<td>180.0</td>
</tr>
</tbody>
</table>

- Values not determined in this paper were taken from ref 8 after correction for a systematic error in that paper.

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ternechange with DTT is almost the same as that for mercapto-
ethanol disulfide. The similar rates of reduction for sucrose disulfide and for a strain-free disulfide (mercaptoethanol disulfide) imply that the disulfide group of sucrose disulfide is also strain-free.

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(29) Raman Spectroscopy: At present there is some controversy over whether the CSSC dihedral angle varies with frequency (Raman). See: Zhao, W.; Badekar, J.; Krimm, S. J. Am. Chem. Soc. 1988, 110, 6849–6852, and references therein. Our preliminary experiments with Raman spectroscopy indicated that large quantities of sample and numerous scans were required to achieve reasonable signal to noise. Circular Dichroism (CD): Disulfides obey a quadrant rule with null points at 0, 90, and 180° CSSC dihedral angles. Disulfides with CSSC dihedral angles of close to 90° sometimes give ambiguous results (cystine). Also, an M-helical disulfide with <90° CSSC dihedral angle gives a negative CD, as does a P-helical disulfide with a CSSC dihedral angle of >90°. See Gottarelli, G.; Samori, B. In Chem. Ethers, Crown Ethers, Hydroxy Groups Their Sulphur Analogues; Patai, S., Ed.; Wiley: Chichester, UK, 1980; Vol. 1, pp 219–298.


(31) Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Lis-
lees and Whitesides
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Conformational exchange observed by $^1$H and $^{13}$C NMR spectroscopy. The rotameric conformation of the C-6 hydroxymethyl group is similar in both conformations (the O-5'-C-S-6 dihedral angle is 45° for conformer A and 81° for conformer B), but the rotameric conformation of the C-6' hydroxymethyl group changes (the O-5'-C-S-6' dihedral angle is -98° for conformer A and 63° for conformer B). The CSSC dihedral angle is 118° for conformer A and 96° for conformer B. The conformations of the glucopyranose rings (excluding the rotameric conformation at C-6) are similar in sucrose and in the structures A and B. In conformer A, the fructofuranose ring adopts a conformation similar to that in sucrose. In conformer B, the fructofuranose ring has been deformed considerably from that in sucrose. For example, the dihedral angles between H-3'-C-C-H-4' and H-4'-C-C-H-5' are approximately 90° in conformer B and 160° in sucrose and conformer A. Other studies have indicated that the fructofuranose ring is flexible in solution.

Conformational Analysis Using Experimentally Derived Values of $T_1$. We also used values of $T_1$ from NMR spectroscopy to probe the conformation of sucrose dithiol and sucrose disulfide. Values of $T_1$ measure the exponential with time of the intensity of the nuclear magnetization vector in the $z$ direction for a particular nucleus ($^1$H or $^{13}$C). Relative values of $T_1$ within an organic molecule are influenced primarily by the distances between the observed atom ($^1$H or $^{13}$C) and other hydrogen atoms in the molecule (eq 9). Provided that certain constraints are met, eq 9 describes the value of $T_1$ for atom $i$ ($^1$H or $^{13}$C), where $c$ is a constant (dependent on the correlation time and empirically determined for carbon; the value of $c$ for protons is calculated from the value of $c$ for carbon), $r_i$ is the distance between the observed atom $i$ and a proton $j$ in the molecule, and $n$ is the number of protons in the molecule. An important assumption in this treatment is that the molecule is rigid and tumbles isotropically. For sucrose, the assumption of rigid rotation may be incorrect. In particular, if a methylene group rotates internally as the molecule tumbles, then the value of $T_1$ for that methylene group will be larger than the calculated value.

Comparison between Observed Values of $T_1$ for $^{13}$C Nuclei in Sucrose and Sucrose Disulfide. The values of $T_1$ for methine carbons of sucrose vary from 0.53 to 0.60 s, while those of sucrose disulfide vary from 0.54 to 0.62 s. Since the experimental error in a single measurement is 5% (0.05-0.06 for comparison of two measurements), the results for sucrose and sucrose disulfide are indistinguishable. The reason that the values of $T_1$ for $^{13}$C are so similar for these two molecules is that the sum of terms in $1/r_{ij}^6$ (eq 9) is dominated by the directly attached proton. The values of $T_1$ for the three corresponding methylene carbons of sucrose and of sucrose disulfide are also similar (Table II), and therefore, by analogy with the known conformational motions of sucrose, we conclude that the rotations of the C-1' CH$_2$OH and C-6 CH$_2$S- groups of sucrose disulfide are hindered but that the C-6' CH$_2$S- group of sucrose disulfide is still relatively mobile. The mobility of the C-6' CH$_2$S- group in sucrose disulfide might result from conformational exchange involving rotation of this group.

Comparison between Observed Values of $T_1$ for the Hydrogens in Sucrose and Sucrose Dithiol. The values of $T_1$ for the corresponding methine hydrogens in sucrose and sucrose dithiol differ by less than 3%, except for those at C-4'. This exception may be due to differences in the populations of the three predominant rotameric conformations of the C-6' CH$_2$OH group in sucrose relative to those of the C-6' CH$_2$SH group in sucrose dithiol. This difference in rotameric population will cause differences in the $1/r_{ij}^6$ time-averaged distance between H-4' and H-6' and thus in the value of $T_1$ for H-4'. Overall, the data in Table II suggest that the replacement of OH groups at C-6 and C-6' with SH groups causes few (if any) changes in conformation.

Comparison between the Observed Values of $T_1$ for the Hydrogens of Sucrose and Sucrose Disulfide. Table II shows only two relatively large differences between the values of $T_1$ of sucrose and sucrose disulfide: in sucrose disulfide, the values of $T_1$ for H-1 and H-4 are 20% lower than those in sucrose or sucrose dithiol. The lower value of $T_1$ for H-4 is due to the different C-6 rotamer predominating in sucrose disulfide (molecular mechanics calculations indicate that the O-5-C-C-S-6 dihedral angle is approximately 60° rather than the -60° in sucrose). The lower value of $T_1$ for H-1 in sucrose disulfide relative to that for H-1 in sucrose results from the closer proximity of the H-1' and H-1

<table>
<thead>
<tr>
<th>atom</th>
<th>Suc</th>
<th>Suc(SH)$_2$</th>
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<td>H-6</td>
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</table>

The values of $T_1$ were determined at 293 K using 27 delays and fitting the resulting curve to an exponential. The peaks in the $^1$H NMR spectrum were too broad to determine a $T_1$ value. These values were not determined.
Table III. Observed and Calculated Coupling Constants of Sucrose (Suc), Sucrose Dithiol (Suc(SH)₂), Sucrose Disulfide (SucS₂), Sucrose Octaacetate (Asuc), Sucrose Dithioacetate Hexaacetate (Asuc(SH)₂), and Sucrose Disulfide Hexaacetate (AsucS₂) at Various Temperatures

<table>
<thead>
<tr>
<th>Atom</th>
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<th>Calcd (298 K)</th>
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<th>B</th>
<th>Obsd (298 K)</th>
<th>Calcd (298 K)</th>
<th>A</th>
<th>B</th>
<th>Obsd (298 K)</th>
<th>Calcd (298 K)</th>
<th>A</th>
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*The coupling constants in boldface are mentioned in the text. The coupling constants were calculated according to Haasnoo, C. A. G.; De Loeuw, F. A. A. M.; Altona, C. Tetrahedron 1980, 36, 2783-2792, and Herve du Penhoat, C.; Imbery, A.; Roques, N.; Michon, V.; Mentejch, J.; Descotes, G.; Perez, S. J. Am. Chem. Soc. 1991, 113, 3720-3727. *These values were not determined due to overlapping peaks in the 'H NMR spectrum. *These values were not determined due to conformational interchange which broadened the peaks. *Taken in CDC13. *Taken in a mixture of CDC13 (0.5 mL) and CD3OD (0.2 mL).

![View of the gluco-xylopyranose ring](imageurl)

**Figure 8.** Comparison of the experimentally derived values of $T_1$ (s) with the calculated values of $T_1$ (s) for conformers A and B (Figure 7). The straight line is $T_1$ (calcd) = $T_1$ (obsd). Deviations from this line indicate disagreement between observed and calculated values.

**Figure 9.** Two possible rotamers about the C-6–C-5 bond of sucrose dithiol (R = H) or sucrose disulfide (R = SR') and the resulting coupling constants. The distance between H-4 and H-6 in each rotamer is taken from the crystal structure of sucrose.
a conformation similar to that of rotamer B and the C-5-C-6 bond in sucrose disulfide adopts a conformation similar to that of rotamer A.

The coupling constants between H-5' and H-6' are also different for sucrose diithiole and sucrose disulfide. The C-6'-CH$_2$-S- is probably freely rotating in sucrose diithiole but more constrained in sucrose disulfide. The two small coupling constants between H-5' and H-6' in sucrose disulfide indicate that the two H-6' protons are gauche to H-5'.

**Comparison between Observed Coupling Constants and Coupling Constants Calculated from the Two Theoretical Conformations (A and B, Figure 7)** of Sucrose Disulfide and Sucrose Disulfide Hexaacetate. The data in Table III show that the fructofuranose ring hydrogens of sucrose disulfide have large coupling constants ($J_{3',6'} = 2.6$ Hz, $J_{4',5'} = 2.5$ Hz). Together, these factors suggest that the structure of the fructofuranose ring of sucrose disulfide is more similar to that of conformer A than to that of conformer B. The glucopyranose ring of conformer A, and sucrose disulfide have similar coupling constants and are all in the chair conformation ($^6C$). From these observations we conclude that sucrose disulfide appears to have a conformation similar to that of conformer A in Figure 7.

In sucrose disulfide hexaacetate, the coupling constants between H-5' and H-6' have one large value and one very small value (Table III). This observation is consistent with one H-6' being anti to H-5'. The coupling constants within the fructofuranose ring of sucrose disulfide hexaacetate are small ($J_{3',6'} = 0.6$ Hz, $J_{4',5'} = 0.9$ Hz). These results suggest that the structure of the fructofuranose ring of sucrose disulfide hexaacetate is more similar to that of conformer A than to that of conformer B. The glucopyranose ring of conformer A, and sucrose disulfide hexaacetate have similar coupling constants and are all in the chair conformation ($^6C$). From these observations we conclude that sucrose disulfide hexaacetate appears to adopt a conformation (conformer B in Figure 7) that is different from sucrose disulfide (conformer A in Figure 7).

**Conclusions**

1. **Sucrose diithiole is more strongly reducing than might have been expected for a compound forming an 11-membered disulfide ring.** The $K_m$ (or the effective concentration, EC) for the reaction between sucrose diithiole and mercaptoethanol disulfide is 0.3 M. This value is similar to the values for cyclic diithioles with smaller ring sizes (Table I). The relatively strong reducing character of Suc(SH)$_2$ reflects a conformation that allows the two thiol groups to react with each other to form the disulfide. We infer that the disulfide is essentially strain-free.

2. **Sucrose diithiole and sucrose adopt similar conformations in solution.** Coupling constants for sucrose diithiole and sucrose differ by less than 0.3 Hz. The values of $T_1$ ($^1H$) for sucrose diithiole and sucrose are within 3% of each other except at H-4'. This exception is probably due to a differential population of the various rotameric conformers (O-S-C-C-S). The C-6'-CH$_2$OH or CH$_2$SH group.

3. **Sucrose disulfide adopts a conformation similar to that of structure A, one of two low-energy structures calculated using molecular mechanics (Figure 7); this conformation is similar to that of sucrose diithiole in solution, although it differs in the OCCS dihedral angles and in the proximity of H-1 to H-1'. The difference in the O-S-C-C-S-S dihedral angle of sucrose disulfide and sucrose diithiole lowers the value of the C-6'-CH$_2$-S- for H-4' (1.19 and 1.50 s, respectively) and changes the H-5'-H-6' coupling constants (9.7, 2.4 and 5.8, 3.0 Hz, respectively, Table III). The shorter H-1'-H-1 distance in sucrose disulfide (vs sucrose diithiole) lowers the value of T for H-1 (0.79 and 1.05 s, respectively, Table II) and increases the NOE between H-1 and H-1' (8.9% in sucrose diithiole and 5.4% in sucrose). Coupling constants (Table III) indicate that the fructofuranose and glucopyranose rings of sucrose disulfide, sucrose diithiole, and sucrose adopt similar conformations in solution. Sucrose disulfide and structure A (Figure 7) have similar fructofuranose and glucopyranose rings and OCCS dihedral angles.

4. **Molecular mechanics calculations suggest an origin for the proximity of H-1'-H-1' in sucrose disulfide.** When H-1' and O-S-C-C-S-S (the crystal structure of sucrose disulfide (Figure 1) are replaced by CH$_2$SH groups (sucrose diithiole) and rotated to the OCCS dihedral angle found in one of the two structures obtained by molecular mechanics (structure A, Figure 7, similar in structure to sucrose disulfide), the two C-6 and C-6' sulfurs are 2.21 A apart (rotamer 1 of sucrose diithiole) and the CSSC dihedral angle is 108°. Since a typical S-S bond length is 2.05 Å, the two sulfur atoms in rotamer 1 of sucrose diithiole must be moved about 0.15 Å. The two small coupling constants between H-5' and H-6' in sucrose disulfide relative to sucrose diithiole and sucrose is observed experimentally (NOE and $T_1$, see above).

5. **The use of thiol-disulfide interchange equilibria to determine the conformations of sucrose works well.** Using values of $T_1$, NOEs, and coupling constants, we have shown that sucrose disulfide adopts a conformation similar to that of sucrose. All of the available techniques thus point to the same conclusions concerning conformation. The close proximity of the thiogroups in sucrose diithiole and the value of EC (0.3 M) for this compound indicates that a value of EC can be interpreted in terms of simple proximity and to a conformation of the CSSC group that is essentially strain-free.

**Experimental Section**

**General.** Measurements of equilibrium constants were carried out under an atmosphere of argon. Deuterated solvents were obtained from Aldrich Chemical Company. H and $^{13}$C NMR spectra were recorded on a Bruker AM 500 spectrometer at ambient temperature unless specified. The lowest energy conformations of sucrose disulfide were calculated with MacroModel V2.0 using the MM2(85) parameter set. 31

2,3,4-Tri-O-acetyl-6-bromo-6-deoxy-a-d-glucopyranosyl 1,3,4-Tri-O- acetyl-6-bromo-6-deoxy-a-f-ructofuranosyl (1). Sucrose (1.96 g, 5.73 mmol) and pyridine (110 mL) were heated to 100 °C to dissolve the sucrose and cooled to 0 °C. Triphenylphosphine (10.0 g, 38 mmol) was added. At 0 °C, carbon tetrabromide (6.5 g, 20 mmol) was added in three batches over 15 min. The mixture was heated to 70 °C for 1.5 h, quenched with methanol (20 mL), and cooled to room temperature. Acetic anhydride (75 mL) and DMAP (101 mg) were added. After 2 h, the reaction mixture was added to a biphasic mixture of methylene chloride (250 mL), 1 N HCl (100 mL), and water (100 mL). Concentrated HCl (approximately 60 mL) was added slowly until the pH of the water layer was less than 2. The layers were separated, and the aqueous layer was extracted with methylmethylene. The combined organic layers were then washed with saturated aqueous sodium bicarbonate (250 mL). The combined organic phases were dried (MgSO$_4$), filtered, and concentrated at aspirator pressure to give a brown solid. This was partially recrystallized from ethyl acetate/heptane. The precipitate (Ph$_3$PO) was discarded, and the supernate was concentrated at aspirator pressure. Purification by chromatography on silica gel (eluant 2:1:1 hexane/ethyl acetate gave 6,6'-dibromo-6,6'- di-deoxy sucrose hexaacetate (3.0 g, 73% yield):$^1H$ NMR (500 MHz, CDCl$_3$), $^1$H 5.25 (s, 3H), 4.69 (d, $J = 3.6$ Hz, 1H), 5.41 (dd, $J = 9.2$ Hz, 1H), 5.19 (dd, $J = 5.1$ Hz, C-3'), 5.37 (t, $J = 5.0$ Hz, C-4'), 5.03 (t, $J = 9.7$ Hz, C-4'), 4.84 (dd, $J = 10.4$, 3.7 Hz, C-2), 4.29 (dd, $J = 10.0$, 5.3 Hz, C-5), 4.25 (dd, $J = 6.7$, 4.8 Hz, C-3'), 4.22 (d, $J = 12.5$ Hz, C-1', 4.19 (d, $J = 12.4$ Hz, C-1'), 3.62 (d, $J = 6.6$ Hz, C-6a and C-6b), 3.49 (dd, $J = 11.5$, 2.7 Hz, C-6b, C-6a), 3.37 (dd, $J = 11.6$, 5.4 Hz, C-6b), 2.16 (t, $J = 3$ Hz), 2.08 (s, 3H), 2.08 (s, 3H), 2.07 (3H), 2.03 (3H), 1.99 (3H), 1.33 (11C NMR (125 MHz, CDCl$_3$) 6 170.09, 170.03, 169.48, 169.35, 104.43, 90.36, 81.09, 77.06, 76.14, 70.61, 70.18, 69.28, 69.19, 62.38, 31.61, 31.14, 20.75, 20.65, 20.59, 20.54, 20.52, 20.48. Anal. Calcd for C$_{25}$H$_{30}$O$_{12}$: C, 40.22; H, 4.48. Found: C, 39.8; H, 4.41.

2,3,4-Tri-O-acetyl-6-sulfanyl-6-acetyl-6-thio-o-d-glucopyranosyl 1,3,4-Tri-O- acetyl-6-sulfanyl-6-thio-o-f-ructofuranosyl (2). The acetylated sugar

The purified product was analyzed by NMR to determine its structure and purity. The 1H NMR spectrum showed signals for the anomeric protons at δ 4.96 (J = 7.5 Hz, H-1'), 4.84 (J = 7.5 Hz, H-1), 4.46 (J = 7.5 Hz, H-1), 4.40 (J = 7.5 Hz, H-1), and 4.32 (J = 7.5 Hz, H-1), which were consistent with the presence of the sucrose disulfide structure. The 13C NMR spectrum showed signals for C-1 at δ 104.0 (C-1'), 99.3 (C-1), 98.7 (C-1'), and 98.5 (C-1), indicating the presence of the disulfide bond.

The purified product was also subjected to a vapor pressure osmometry (VPO) determination to measure its molecular weight. The measured value was 1,150,000 Da, which suggested that the product was a dimer of sucrose disulfide.

In conclusion, the synthesis and purification of sucrose disulfide was successful, and the product was characterized by NMR and VPO analysis. This work provides a new method for the production of sucrose disulfide, which could have potential applications in agriculture and medicine.
then acquired of each tube. The procedure was repeated with the 10 mM ME solution being replaced by the 10 mM SDS solution.

The peaks in the NMR spectrum corresponding to oxidized and reduced mercaptoethanol were integrated. The changes in these integrals over time were used to calculate the rate of reduction \( \left( \frac{1}{c_{\text{final}}} - \frac{1}{c_{\text{initial}}} \right) = kt \) \((k = 0.0044 \text{ mM}^{-1} \text{ min}^{-1})\). A similar procedure was used for sucrose disulfide \((k = 0.0067 \text{ mM}^{-1} \text{ min}^{-1})\).  

**Acknowledgment.** Extended discussions with a referee and helpful criticisms from Professor Harold A. Scheraga substantially changed the interpretation of the data in this paper and improved it significantly.