Chair–chair conformational flexibility, pseudorotation, and exocyclic group isomerization of monosaccharides in water

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(Received 3 October 2001; accepted 26 November 2001)

Acoustical absorption spectra between 10 kHz and 2 GHz are reported for various monosaccharides in water. With the exception of solutions of methyl-β-D-arabinopyranoside (0.5 mol/l) the spectra reveal absorption with relaxation characteristics in excess to the asymptotic high frequency absorption term. Up to three relaxation terms per spectrum emerge within the measuring frequency range. Regression analysis of the measured spectra in terms of a suitable analytical spectral function yields five relaxation regimes with relaxation times on the order of 1 μs, 100 ns, 1 ns, or 100 ps, respectively. These relaxation regimes are assigned to the chair–chair ring inversion, two modes of pseudorotation, an exocyclic side group isomerization and a molecular association mechanism. Particular emphasis is given to the ring inversion which is additionally verified by time resolved measurements of nonequilibrium tautomer systems, utilizing the coupling of the inversion to the carbohydrate mutarotation. Further evidence is derived from measurements of solutions of D-fructose in mixtures of ethanol and water. © 2002 American Institute of Physics.

DOI: 10.1063/1.1436123

INTRODUCTION

Carbohydrates hold a key position in the molecular logic of life. Contributing to almost all forms of life on earth, this class of molecules constitutes most of the organic matter in the biosphere. Carbohydrates do not just provide the main energy resource of living matter, used to maintain the structural and functional properties of biological cells, they are structural and functional elements themselves, and, due to various specific characteristics, participate in many biochemical reactions.

The complex functions of carbohydrate molecules in biology may be related to the accumulation of functional groups, predominantly hydroxy group, the concentration of which is higher than with any other class of biomolecules. Though carbohydrates provide specific¹ and unusual²–⁴ interactions with water, little attention has been directed toward the solution properties of mono- and oligosaccharides in the past,⁵,⁶ probably because of the belief that their chemistry and biology has already been fully established.⁷ More recently, the study of carbohydrates has greatly enriched biochemistry and physicochemistry, particularly by focusing on the outstanding conformational variety and flexibility of these molecules. Due to their potential to form a multitude of conformers saccharides are considered key elements in a biochemical alphabet, beyond the genetic code, assumed to play a fundamental, but still not completely understood, role in intra- and intercellular recognition processes.⁸–¹⁰

Many interrelations between conformational equilibria and the molecular structure of carbohydrates have been studied utilizing a variety of available methods.¹¹ Special emphasis has been given to NMR spectroscopy,¹²,¹³ and molecular dynamics simulations.¹⁴–¹⁸ Acoustical absorption spectrometry offers another experimental approach for the direct monitoring of dynamic processes.¹⁹ This method is well established now for measurements in wide frequency range,²⁰ corresponding with the relaxation time regime between about 20 μs and 20 ps, which is difficult to attain by other techniques. Coupling to changes in the molar volume and enthalpy, sonic fields probe the native system to be studied. Additionally, acoustic spectrometry takes advantage from its high sensitivity, enabling almost all molecular processes in liquids to be investigated in detail. For these reasons, some ultrasonic relaxation measurements, aiming at the kinetics of carbohydrate conformational changes in water, have been reported in the past.²¹–²⁴ The frequency range of those early studies, however, was too limited to account accordingly for the complex structure in the ultrasonic spectra of saccharide solutions.

Recent acoustical measurements of aqueous solutions of various monosaccharides,²⁵,²⁶ performed in an extended frequency range, revealed five relaxation regions. The relaxation processes were tentatively assigned to different solute conformational equilibria as well as a solute–solute association mechanism. In order to confirm those assignments we report here acoustical spectra of monosaccharide solutions that have been measured over an even broader frequency range (10 kHz ≤ ν ≤ 2 GHz; ν, frequency of measurements), thus allowing for a full characterization of the suggested chair–chair ring isomerization of some carbohydrates. This isomerization process is important for stereochemistry in general. It is also assumed to control the complexation of carbohydrates with cations, such as the biochemically relevant calcium ion. Therefore, we particularly focus on the chair–chair conformational isomerization in this article. Aiming at a comprehensive evaluation of the broadband ultrasonic spectra, however, the other elementary molecular
processes, coupling to the (compressional) acoustic waves, will be also briefly discussed.

**MATERIALS AND METHODS**

**Monosaccharide solutions**

A survey of the monosaccharides included in this study is given in Fig. 1. For completeness, also given in that figure are saccharides for which ultrasonic spectra have already been reported previously. Some of these previous spectra have now been complemented at low frequencies (υ < 100 kHz) and have been reevaluated afterward. For simplicity, only the dominating conformation of each monosaccharide is depicted in Fig. 1, except D-fructose, for which the four tautomers are shown as an example. The equilibrium mole fractions of the carbohydrate tautomers in aqueous solution are given in Table I.

With the exception of D-mannose (98%), D-galactose (98%), and 1,6-anhydro-β-D-glucopyranoside (98%), the purity of the carbohydrates was 99% or better. All carbohydrates have been used as delivered by the manufacturers (SIGMA, FLUKA; Deisenhofen, Germany), but have been dried at 60°C for at least 12 h under reduced pressure before sample preparation. Water was doubly distilled, deionized, and UV sterilized. Solutions were prepared in volumetric flasks by weighing the monosaccharide and adding water up to the line measure. All solutions were first stored for a minimum of 15 h in order to allow for the complete establishment of the tautomer equilibrium before starting measurements.

Some parameters of the monosaccharide solutions are given in Table II. The density ρ of the samples has been determined using a pycnometer that had been calibrated against distilled and degassed water. The shear viscosity ηs of the solutions has been measured with the aid of a falling ball viscometer (B/BN, Haake, Karlsruhe, Germany) that had been calibrated using standard reference liquids supplied by the manufacturer. Also presented in Table II is the sound velocity cs of the solutions. The cs values have been determined at frequencies υ < 15 MHz from the resonance frequencies of successive principal resonance peaks of cavity resonator cells, filled with the sample liquid. In those measurements nonequidistancy of the resonance frequencies has been carefully taken into account.

**Broadband acoustical spectrometry**

Acoustical absorption spectra α(υ) of liquids are usually described in terms of two different contributions:

\[
α(υ) = B'υ^2 + α_{exc}(υ),
\]

with \( B'υ^2 \) representing a background part in the total absorption coefficient α and with \( α_{exc} \) denoting the excess contributions that might be due to elementary molecular reactions. Within our frequency range of measurements (10^4 Hz ≤ υ ≤ 2 × 10^9 Hz), the background term in the absorption coefficient [Eq. (1)] changes by the factor 4 × 10^7. In order to reach a sufficiently high accuracy over this range of α values and to also account for the broad range of wavelengths \( λ = c_s/υ \) in the measurements (750 nm ≤ λ ≤ 75 mm) two different experimental methods have been applied. Altogether eight different sample cells have been used in the measurements; each cell was matched to a particular frequency range.

At low frequencies (υ < 15 MHz) we applied cavity resonator methods in which the resonator cells was completely filled with the sample liquid. The pathway of acoustical wave interaction with the liquid under test was significantly enhanced by multiple reflections. Below 150 kHz a radial mode spherical resonator was appropriate. In addition, three cells were operated in a quasiplane wave mode. They were designed to account for the comparatively small α_{exc} of the saccharide solutions and to thus reduce undesired diffraction losses that result from the finite cell diameter. We used a biconev cell (effective transducer diameter \( 2r_T = 91 \text{ mm}; \) effective cell length \( l = 19 \text{ mm} \)) at 80 kHz ≤ υ ≤ 1.3 MHz. Between 100 kHz and 2.7 MHz a planoconcave resonator \( (2r_T = 70 \text{ mm}; \) radius of curvature \( R_C = 2 \text{ m}; \) \( l = 19 \text{ mm} \)) was utilized and between 800 kHz and 15 MHz a biplanar cell \( (2r_T = 16.8 \text{ mm}; \) \( l = 6 \text{ mm} \)). To properly account for higher-order acoustical field modes within the cavity resonator we always measured the complete transfer function around a principal resonance peak of interest and interest.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>α-pyranose</th>
<th>β-pyranose</th>
<th>α-furanose</th>
<th>β-furanose</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-arabinose</td>
<td>0.60</td>
<td>0.355</td>
<td>0.025</td>
<td>0.020</td>
</tr>
<tr>
<td>D-xylose</td>
<td>0.365</td>
<td>0.63</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D-lyxose</td>
<td>0.70</td>
<td>0.28</td>
<td>0.015</td>
<td>0.005</td>
</tr>
<tr>
<td>D-ribose</td>
<td>0.215</td>
<td>0.585</td>
<td>0.065</td>
<td>0.135</td>
</tr>
<tr>
<td>D-galactose</td>
<td>0.30</td>
<td>0.64</td>
<td>0.025</td>
<td>0.035</td>
</tr>
<tr>
<td>D-glucose</td>
<td>0.38</td>
<td>0.62</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td>D-mannose</td>
<td>0.637</td>
<td>0.355</td>
<td>0.006</td>
<td>0.002</td>
</tr>
<tr>
<td>D-fructose</td>
<td>0.02</td>
<td>0.70</td>
<td>0.05</td>
<td>0.23</td>
</tr>
<tr>
<td>L-sorbose</td>
<td>0.98</td>
<td>0</td>
<td>0.02</td>
<td>0</td>
</tr>
</tbody>
</table>
obtained the desired resonance frequency and quality factor from a multipoint fit of the scan to the suitable analytical expression for the transfer function. Reference measurements have been performed with liquids of carefully adjusted sound velocity \( c_s \) and density \( \rho \) in order to appropriately correct the measured quality factor for intrinsic cell losses.\(^\text{29-35}\) We used water/methanol mixtures and aqueous solutions of urea with well-known absorption coefficient as reference liquids.\(^\text{29-35}\) Measurements with liquids of carefully adjusted sound velocity \( c_s \) and density \( \rho \) have been performed by transmitting pulse-modulated sonic waves through a cell of variable sample length \( l \), and by measuring the transfer function \( T(l) \) at frequencies below 30 MHz. These effects have been taken into account using a semiempirical correction term,\(^\text{34}\) obtained from calibration measurements in which suitable reference liquids were used.

**Experimental errors**

With the resonator measurements, the main sources of possible experimental errors are small disturbances in the cell geometry and cell adjustment that might result from the cleaning and refilling procedure when the sample is exchanged for the reference liquid. Imperfect wetting of the transducer surfaces, covered with a gold layer, may also affect the resonance peaks. The \( \alpha \) values obtained from the pulse-modulated traveling wave transmission technique may be subject to an incomplete parallelism of the transmitter and the transducer area is finite, diffraction effects may affect the cell transfer function at frequencies below 30 MHz. These effects have been taken into account using a semiempirical correction term,\(^\text{34}\) obtained from calibration measurements in which suitable reference liquids were used.

**TABLE II. Molar concentration \( c \) and molarity \( m \) of solute, density \( \rho \), shear viscosity \( \eta_s \), and sound velocity \( c_s \) of the liquid for aqueous solutions of carbohydrates.**

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>25°C ±0.05 K</th>
<th>( c )/mol l(^{-1} ) ±0.2%</th>
<th>( m )/mol kg(^{-1} ) ±0.1%</th>
<th>( \rho /\text{g cm}^{-3} ) ±0.2%</th>
<th>( 10^7 \eta /\text{Pa s} ) ±2%</th>
<th>( c_s /\text{mol l}^{-1} ) ±0.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-arabinose</td>
<td>1.08</td>
<td>1.99</td>
<td>1.061</td>
<td>1.37</td>
<td>1566.5</td>
<td></td>
</tr>
<tr>
<td>D-xylose</td>
<td>1.07</td>
<td>1.99</td>
<td>1.055</td>
<td>1.41</td>
<td>1555</td>
<td></td>
</tr>
<tr>
<td>D-lyxose</td>
<td>1.08</td>
<td>1.99</td>
<td>1.064</td>
<td>1.53</td>
<td>1560</td>
<td></td>
</tr>
<tr>
<td>D-ribose</td>
<td>1.07</td>
<td>1.99</td>
<td>1.056</td>
<td>1.41</td>
<td>1553</td>
<td></td>
</tr>
<tr>
<td>D-galactose</td>
<td>1.00</td>
<td>1.129</td>
<td>1.069</td>
<td>1.57</td>
<td>1570</td>
<td></td>
</tr>
<tr>
<td>D-glucose</td>
<td>0.50</td>
<td>0.534</td>
<td>1.031</td>
<td>1.12</td>
<td>1526</td>
<td></td>
</tr>
<tr>
<td>D-mannose</td>
<td>1.00</td>
<td>1.130</td>
<td>1.065</td>
<td>1.47</td>
<td>1563</td>
<td></td>
</tr>
<tr>
<td>D-fructose</td>
<td>0.50</td>
<td>0.533</td>
<td>1.032</td>
<td>1.15</td>
<td>1531</td>
<td></td>
</tr>
<tr>
<td>D-glucose</td>
<td>0.70</td>
<td>0.778</td>
<td>1.046</td>
<td>1.27</td>
<td>1545</td>
<td></td>
</tr>
<tr>
<td>L-sorbose</td>
<td>1.00</td>
<td>1.134</td>
<td>1.066</td>
<td>1.47</td>
<td>1567.5</td>
<td></td>
</tr>
<tr>
<td>D-fructose</td>
<td>1.50</td>
<td>1.735</td>
<td>1.101</td>
<td>1.83</td>
<td>1606</td>
<td></td>
</tr>
<tr>
<td>L-sorbose</td>
<td>1.00</td>
<td>1.134</td>
<td>1.065</td>
<td>1.47</td>
<td>1568</td>
<td></td>
</tr>
<tr>
<td>Methyl-( \beta )-D-arabinopyranoside</td>
<td>1.1</td>
<td>1.300</td>
<td>...</td>
<td>...</td>
<td>1578</td>
<td></td>
</tr>
<tr>
<td>Methyl-( \beta )-D-xylopyranoside</td>
<td>0.56</td>
<td>0.607</td>
<td>1.021</td>
<td>1.17</td>
<td>1537</td>
<td></td>
</tr>
<tr>
<td>Methyl-( \beta )-D-glucopyranoside</td>
<td>1.13</td>
<td>1.300</td>
<td>1.051</td>
<td>1.60</td>
<td>1571</td>
<td></td>
</tr>
<tr>
<td>Methyl-( \beta )-D-xylopyranoside</td>
<td>1.48</td>
<td>1.800</td>
<td>1.067</td>
<td>2.01</td>
<td>1600</td>
<td></td>
</tr>
<tr>
<td>1,6-anhydro-( \beta )-D-glucopyranoside</td>
<td>0.47</td>
<td>0.500</td>
<td>1.025</td>
<td>1.20</td>
<td>1532</td>
<td></td>
</tr>
<tr>
<td>1,6-anhydro-( \beta )-D-glucopyranoside</td>
<td>0.47</td>
<td>0.500</td>
<td>1.026</td>
<td>1.04</td>
<td>1517</td>
<td></td>
</tr>
</tbody>
</table>

\[\text{FIG. 2. Ultrasonic absorption spectra in the format } \alpha /\nu^2 vs \nu \text{ for 0.5 mol/l solutions of D-glucose (●) and of 1,6-anhydro-\( \beta \)-D-glucopyranoside (○) in water at 25°C. The dashed line indicates the frequency-independent } \alpha /\nu^2 (=B') \text{ value for water at the same temperature.}\]
receiver transducer units, and at low frequencies, to insufficient corrections for diffraction losses. Strictly, the experimental errors of the absorption data depend on the $\alpha$ values themselves. Approximately their values are those given below: $\Delta \alpha/\alpha = 0.5, 10-20$ kHz; $\Delta \alpha/\alpha = 0.15, 20-100$ kHz; $\Delta \alpha/\alpha = 0.1, 0.1-3$ MHz; $\Delta \alpha/\alpha = 0.02, 3-25$ MHz; $\Delta \alpha/\alpha = 0.01, 25-300$ MHz; $\Delta \alpha/\alpha = 0.03, 0.3-1$ GHz; $\Delta \alpha/\alpha = 0.01, 1-2$ GHz. The temperature $T$ of the specimen cells was controlled to within $\pm 0.03$ K, and it was measured with an accuracy of $\pm 0.02$ K. Temperature gradients within a cell and differences in the temperature of different cells did not exceed $0.05$ K, corresponding to an error of less than $0.001$ in the $\alpha$ data of the samples. Fluctuations $\Delta \alpha/\alpha$ in the frequency of the acoustical signals were smaller than $0.0001$ and can thus be omitted here.

RESULTS AND ANALYTICAL REPRESENTATION OF SPECTRA

In Fig. 2, a frequency-normalized representation of the acoustical spectra of a 0.5 molar aqueous solution of D-glucose and 1,6-anhydro-\beta-D-glucose are shown. Within the frequency range of measurements, the frequency normalized data of the latter solution are constant, $a^2/\nu^2 = B^2$, indicating the absence of excess absorption [Eq. (1)] in the bicyclic carbohydrate system. The spectrum of the glucose solution clearly reveals excess absorption with relaxation characteristics $d(a/\nu^2)/d\nu<0$ at high frequencies. In Fig. 3 spectra for 1 molar solutions of the aldopentoses D-arabinose and D-ribose are shown. In order to accentuate the high-frequency part of the spectra, the data are presented in the format

\[
(\alpha/\nu^2) c_\nu = \alpha \lambda = (\alpha \lambda)_{\text{exc}} + B \nu,
\]

where $B = B' c_s$. Calculating the absorption-per-wavelength data frequency-independent sound velocities (Table II) have been used. This is justified because the dispersion in $c_s$, corresponding with the excess absorption, is small throughout.

The acoustical spectra for both aldopentose solutions show three relaxation regions $[(\alpha \lambda)_{\text{exc}} > 0]$, as illustrated for the relaxation terms of the D-arabinose spectrum by dashed curves in Fig. 3. The excess absorption spectra for solutions of the keto-hexoses D-fructose and L-sorbose, respectively, also display a superposition of relaxation terms but exhibit substantially different characteristics at low frequencies (Fig. 4).

Careful inspection of all measured spectra showed that the frequency-dependent excess absorption per wavelength of the carbohydrate solutions can be adequately represented by a sum of up to three relaxation spectral terms with discrete relaxation times $\tau_n$ (n = 1,...,3). Therefore, the experimental $\alpha \lambda$ spectra have been analytically described by the function

\[
R_\alpha(\nu) = \sum_{n=0}^{N} A_n \frac{\omega}{\omega^n} \frac{\tau_n}{\tau_n^2} + B \nu, \tag{3}
\]

with $N = 0, 1, 2, 3$. The $A_n$, $n = 1,...,3$, denote relaxation amplitudes, $A_0 = 0$, and $\omega = 2 \pi \nu$. $R_\alpha(\nu)$ has been fitted to the measured spectra using a nonlinear least-squares regression analysis to minimize the variance

\[
\chi^2 = \frac{1}{I - J - 1} \sum_{I,J} w^2(\nu_i) \left[\frac{\alpha(\nu_i)c_\nu}{\nu_i - R(\nu_i, P_j)}\right]^2, \tag{4}
\]

TABLE III. Parameters of the relaxation spectral function defined by Eq. (3) for aqueous solutions of aldopentoses at 25°C.

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>$c$/mol l$^{-1}$</th>
<th>$A_1$ $10^{-3}$</th>
<th>$\tau_1/\mu$s</th>
<th>$A_2$ $10^{-3}$</th>
<th>$\tau_2/\mu$s</th>
<th>$A_3$ $10^{-3}$</th>
<th>$\tau_3/\mu$s</th>
<th>$B$/ps</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-arabinose</td>
<td>1.08</td>
<td>0.20</td>
<td>0.54</td>
<td>0.07</td>
<td>10</td>
<td>0.50</td>
<td>0.4</td>
<td>37.4</td>
</tr>
<tr>
<td>D-xyllose</td>
<td>1.07</td>
<td>...</td>
<td>...</td>
<td>0.04</td>
<td>40</td>
<td>...</td>
<td>0.43</td>
<td>38.0</td>
</tr>
<tr>
<td>D-lyxose</td>
<td>1.08</td>
<td>0.88</td>
<td>1.30</td>
<td>0.12</td>
<td>7.2</td>
<td>0.19</td>
<td>0.6</td>
<td>38.2</td>
</tr>
<tr>
<td>D-ribose</td>
<td>1.07</td>
<td>1.06</td>
<td>1.71</td>
<td>0.13</td>
<td>3.5</td>
<td>2.0</td>
<td>0.1</td>
<td>37.1</td>
</tr>
</tbody>
</table>
where the $\alpha(n, c_i, \nu_j, n_i, c_j, \nu)$, $i = 1, ..., J$, are the total absorption-per-perservation data, measured at frequencies $\nu_j$, and the $\omega(n, c_i, \nu_j, n_i, c_j, \nu)$ are weighing factors, set inversely proportional to the experimental uncertainties $\Delta \alpha(n, c_i, \nu_j)$ for each $P_j$, $i = 1, ..., J$ are the adjustable parameters of the model relaxation function, $J$ is the number of frequencies of measurements, and $J = 2N + 1$ is the number of free parameters of $R_n$.

Inspection of the complete set of experimental data, in fact, indicated a maximum of three relaxation terms per spectrum. These terms, however, extend over five different relaxation regions, with relaxation frequencies $\nu(n, c_i, \nu_j, n_i, c_j, \nu)$ at 25°C between 0.01 and 4 MHz ($\alpha$), 0.9 and 4 MHz ($\beta$), 10 and 40 MHz ($\gamma$), 70 and 300 MHz ($\delta$), as well as 200 and 2000 MHz ($\epsilon$). In conformity with the previous notation, the parameter values (Tables III–VII) are ordered in terms of these relaxation regions $\alpha$ to $\epsilon$.

**DISCUSSION**

Substantially different sonic absorption spectra result for the aqueous solutions, even if the monosaccharides differ from one another only by fine details of their structures, as, for example, the axial/equatorial direction of just one hydroxy group. Quite remarkably, binding of a methyl group to the anomeric OH-group of D-arabinose eliminates the three relaxation terms

monosaccharides, the $1C_i$ conformational equilibrium of methyl cyclohexane is much larger ($\approx 6.9$ kJ/mol, $\alpha$-D-xylene), thus clearly favoring one chair conformation. Consequently, their spectra do not reveal an $\alpha$ term.

Another confirmation of our assignment of the $\alpha$-relaxation term to the chair–chair conformational equilibrium follows from time-resolved nonequilibrium measurements of the sonic absorption of carbohydrate aqueous solutions. According to the interaction energy differences $\Delta U$ (Table VIII) only one pyranose anomer of D-arabinose, D-lyxose, and D-fructose, respectively, is capable of a $1C_i$ conformational equilibrium. For D-arabinose solutions in equilibrium, the relative content of this ($\beta$-anomer is 35.5%. With solutions of D-fructose in water the chair–chair conformational change is possible only for the small content of 2% $\alpha$-anomers. In crystalline form, both monosaccharides are available as 100% $\beta$-pyranose tau-

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>$c$/mol l$^{-1}$</th>
<th>$A_\alpha/10^{-3}$</th>
<th>$\tau_\alpha/\mu$s</th>
<th>$A_\beta/10^{-3}$</th>
<th>$\tau_\beta/ps$</th>
<th>$A_\gamma/10^{-3}$</th>
<th>$\tau_\gamma/\mu$s</th>
<th>$A_\delta/10^{-3}$</th>
<th>$\tau_\delta/\mu$s</th>
<th>$A_\epsilon/10^{-3}$</th>
<th>$\tau_\epsilon/\mu$s</th>
<th>$B$/ps</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-fructose</td>
<td>0.5</td>
<td>0.32</td>
<td>1.19</td>
<td>0.32</td>
<td>1.19</td>
<td>0.32</td>
<td>1.19</td>
<td>0.32</td>
<td>1.19</td>
<td>0.32</td>
<td>1.19</td>
<td>0.32</td>
</tr>
<tr>
<td>D-glucose</td>
<td>0.7</td>
<td>0.44</td>
<td>1.13</td>
<td>0.44</td>
<td>1.13</td>
<td>0.44</td>
<td>1.13</td>
<td>0.44</td>
<td>1.13</td>
<td>0.44</td>
<td>1.13</td>
<td>0.44</td>
</tr>
<tr>
<td>D-mannose</td>
<td>1.0</td>
<td>0.71</td>
<td>1.23</td>
<td>0.71</td>
<td>1.23</td>
<td>0.71</td>
<td>1.23</td>
<td>0.71</td>
<td>1.23</td>
<td>0.71</td>
<td>1.23</td>
<td>0.71</td>
</tr>
<tr>
<td>L-sorbose</td>
<td>1.5</td>
<td>1.02</td>
<td>1.37</td>
<td>1.02</td>
<td>1.37</td>
<td>1.02</td>
<td>1.37</td>
<td>1.02</td>
<td>1.37</td>
<td>1.02</td>
<td>1.37</td>
<td>1.02</td>
</tr>
</tbody>
</table>

**TABLE IV. Parameters of the relaxation spectral function defined by Eq. (3) for aqueous solutions of aldohexoses at 25°C.**
TABLE VI. Parameters of the relaxation spectral function defined by Eq. (3) for a 1.5 mol/l solution of D-fructose in water.

<table>
<thead>
<tr>
<th>T°/C ±0.05 K</th>
<th>A_a/10^{-3} ±5%</th>
<th>\tau_a/\mu s ±5%</th>
<th>A_s/10^{-3} ±10%</th>
<th>\tau_s/\mu s ±10%</th>
<th>A_d/10^{-3} ±10%</th>
<th>\tau_d/\mu s ±10%</th>
<th>B/ps ±30% ±30% ±4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.82</td>
<td>2.22</td>
<td>2.92</td>
<td>2.53</td>
<td>2.2</td>
<td>0.30</td>
<td>66.8</td>
</tr>
<tr>
<td>20</td>
<td>1.08</td>
<td>2.09</td>
<td>2.87</td>
<td>2.14</td>
<td>2.4</td>
<td>0.17</td>
<td>56.0</td>
</tr>
<tr>
<td>25</td>
<td>1.02</td>
<td>1.37</td>
<td>2.89</td>
<td>1.38</td>
<td>4.3</td>
<td>0.17</td>
<td>46.4</td>
</tr>
<tr>
<td>30</td>
<td>1.08</td>
<td>1.00</td>
<td>1.80</td>
<td>1.60</td>
<td>1.7</td>
<td>0.6</td>
<td>40.2</td>
</tr>
<tr>
<td>35</td>
<td>1.05</td>
<td>0.71</td>
<td>3.17</td>
<td>0.99</td>
<td>...</td>
<td>...</td>
<td>35.4</td>
</tr>
</tbody>
</table>

Thus,

\[ Y = Y^* \] (5)

this situation offers the acoustical study of the pyranose another equilibrium \( Y = Y \) following the development of the equilibrium \( Y = Y^* \) between the chair–chair ring conformers. In the above argumentation it has been tacitly taken into account that the latter equilibrium is characterized by much larger rate constants than the former one, which requires breakage and reformation of a covalent bond of the cyclic molecules. From optical activity measurement it is known that this breakage and reformation (mutarotation) occurs on a time scale of hours so that the \( Y = Y^* \) ring isomerization can, in fact, be considered in equilibrium during the mutarotation. The relaxation time related to the unimolecular \( ^{1}C_j=^{1}C_i \) reaction should be independent of the relevant pyranose concentration \( \{ Y \} = \{ Y \} + \{ Y^* \} \). Hence \( \alpha \kappa_s \) data, measured at a fixed frequency \( \nu^s \) within the frequency range of the \( \alpha \) relaxation, are expected proportional to the relaxation amplitude \( A_a \) and thus to the tautomer concentration \( \{ Y \} \), because, for a process of this type, the relaxation amplitude depends on the isentropic molar volume change \( \Delta V_s \) as

\[ A_a = \frac{\pi}{\kappa_s RT} \Gamma \Delta V_s^2, \] (6)

with the isentropic compressibility \( \kappa_s = \rho c_s^2 \) and the stoichiometric factor \( \Gamma \), given by the relation

\[ \Gamma^{-1} = \left[ ^{1}C_j \right]^{-1} + \left[ ^{1}C_i \right]^{-1}; \] (7)

Thus,

\[ \Gamma = [\{ Y \}] K_a / (1 + K_a)^2. \] (8)

Here \( K_a = [\{ C_i \}] / [\{ C_j \}] \) denotes the equilibrium constant of the chair–chair ring inversion. As shown by Fig. 6, time-resolved acoustical absorption measurements\(^{43}\) of freshly prepared D-fructose and D-arabinose solutions at fixed frequency \( \nu^s = 380 \) kHz, in fact, revealed an exponential increase of the \( \alpha \)-D-fructopyranose concentration \( \{ Y \} = \{ Y \} + \{ Y^* \} \) and an exponential decrease of the \( \beta \)-D-arabinopyranose concentration \( \{ \hat{Y} \} \). The time-dependent data clearly reflect the mutarotation of the carbohydrates.\(^{45}\) Additionally, they mean a strong indication of the \( \alpha \)-relaxation term to reflect the \( ^{1}C_j=^{1}C_i \) inversion.

In Fig. 7, a further confirmation of the assignment of the \( \alpha \)-relaxation term to the ring inversion is presented. The diagram shows the acoustical excess absorption spectra for solutions of 0.5 mol/l D-fructose in water and also in a water/ethanol mixture with mole fraction \( x_e = 0.81 \) of the alcohol. Obviously, the low-frequency \( \alpha \)-relaxation term hardly exists in the spectrum for the alcohol-rich system, in conformity with the prediction\(^{45}\) of a vanishing \( \alpha \)-D-fructopyranose content at mole fractions \( x_e \) between 0.75 and 0.9.

Let us inspect the dependence of the relaxation time \( \tau_a \) of the 1.5 mol/l D-fructose solution (Table VI) upon temperature now. For a unimolecular reaction, the relaxation rate \( \tau_a^{-1} \), according to the simple relation

\[ \tau_a^{-1} = k_f^a + k_r^a, \] (9)

is given by the forward \( (k_f^a) \) and reverse \( (k_r^a) \) rate constants, with \( k_f^a = k_r^a \). Eyring theory\(^{46}\) yields

\[ \tau_a^{-1} = k^a (1 + K_a) = \frac{kT}{h} (1 + K_a) \exp \left( \frac{\Delta S^a}{R} \right) \exp \left( -\frac{\Delta H^a}{RT} \right), \] (10)

TABLE VII. Parameters of the relaxation spectral function [defined by Eq. (3)] for aqueous solutions of monosaccharide derivatives at 25 °C.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>( c/\text{mol}^{-1} ) ±0.2%</th>
<th>( A_a/10^{-3} ) ±10%</th>
<th>( \tau_a/\mu s ) ±10%</th>
<th>( A_s/10^{-3} ) ±10%</th>
<th>( \tau_s/\mu s ) ±10%</th>
<th>( A_d/10^{-3} ) ±10%</th>
<th>( \tau_d/\mu s ) ±10%</th>
<th>( B/\text{ps} ) ±30% ±30% ±4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl-( \beta )-D-arabinopyranoside</td>
<td>1.1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Methyl-( \beta )-D-xylopyranoside</td>
<td>0.56</td>
<td>0.10</td>
<td>70</td>
<td>...</td>
<td>...</td>
<td>0.54</td>
<td>0.69</td>
<td>34.6</td>
</tr>
<tr>
<td></td>
<td>1.13</td>
<td>0.27</td>
<td>51</td>
<td>0.28</td>
<td>1.2</td>
<td>1.23</td>
<td>0.36</td>
<td>38.7</td>
</tr>
<tr>
<td></td>
<td>1.48</td>
<td>0.36</td>
<td>57</td>
<td>0.50</td>
<td>1.3</td>
<td>1.80</td>
<td>0.35</td>
<td>56.6</td>
</tr>
<tr>
<td>Methyl-( \beta )-D-glucopyranoside</td>
<td>0.47</td>
<td>...</td>
<td>1.51</td>
<td>1.7</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>35.4</td>
</tr>
<tr>
<td>1,6-anhydro-( \beta )-D-glucopyranoside</td>
<td>0.50</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>33.7</td>
</tr>
</tbody>
</table>
with the entropy $\Delta S^\#_r$ and enthalpy $\Delta H^\#_r$ of activation of the reverse reaction and with the usual meaning of the other symbols. It follows that a plot of $\ln(h\tau_{eq}^{-1}/kT)$ as a function of $1/T$ is linear if the reaction enthalpy $\Delta H_0$ is small compared to $\Delta H^\#_r$, so that $\Delta H^\#_r \approx \Delta H^\#_f$, and if $K_2$, $\Delta S^\#_r$, and $\Delta H^\#_r$ are independent of $T$. As shown by Fig. 8, a logarithmic plot of $\tau_a/1/T$ versus $1/T$ reveals a linear relationship. Its slope is determined by the relation

$$\frac{d\ln\left(\frac{h\tau_{eq}^{-1}}{kT}\right)}{d(1/T)} = \frac{\Delta H^\#_f}{R} - \frac{K_f}{1 + \frac{\Delta H^\#_f}{R}}. \quad (11)$$

For reasons of simplification, let us assume the free energy change $\Delta G_0$ associated with the chair–chair isomerization to be given by the interaction enthalpy difference (Table VIII),

$$\Delta G_0 \approx \Delta H_0 \approx \Delta U, \quad (12)$$

so that the equilibrium constant of the reaction may be calculated from the van’t Hoff relation as

$$K_a = \exp(-\Delta U/RT). \quad (13)$$

Using $\Delta U = 2.7$ kJ/mol (Table VIII), $K_a = 0.34$ results, indicating that the second term on the right-hand side of Eq. (11) is small as compared to the first term. The slope of the regression line in Fig. 8 yields $\Delta H^\#_{f-1} = (42 \pm 4)$ kJ/mol, in agreement with the value for the $^2C_5 = ^3C_2$ conformational isomerization given in the literature. 28

Along with Eqs. (7) and (12), Eq. (6) allows us to calculate the isentropic volume change,

$$\Delta V_s = \Delta V - \frac{\Delta U}{\rho c_p} \Delta H_0. \quad (14)$$

associated with the ring inversion. In Eq. (14), $\Delta V$ is the isothermal volume change, $a_p = [\partial \ln(V)/\partial T]_p$ is the thermal expansion coefficient at constant pressure, and $c_p$ is the heat capacity at constant pressure. The isentropic volume changes resulting from the relaxation amplitudes $A_a$ of 1 molar solutions are compiled in Table VIII. Using again $\Delta H_0 = \Delta U$, the reaction enthalpy term in Eq. (14) can be calculated. It is found on the order of 0.1 ml/mol so that $\Delta V \approx \Delta V_s$ throughout. The relaxation amplitudes of the solutions with different D-fructose concentration yield quite similar volume changes (6.7 ml/mol $\leq \Delta V_s \leq 7.5$ ml/mol, 0.5 mol/l $\leq c \leq 1.5$ mol/l), pointing to consistency in the evaluation of data.

Reasonable reasonable volumes result from the acoustical absorption spectra. For the volume change associated with the exocyclic-CH$_2$OH-group rotation of D-glucose, for example, $\Delta V_s = 2$ ml/mol was found. 25 Interesting, the volume change associated with the conformational isomerization of the aldopentoses is distinctly smaller (0.9 ml/mol $\leq \Delta V_s \leq 1.4$ ml/mol, Table VIII) than that of the fructopyranose. The ring inversion of the ketohexose involves the rotational change relative to the ring of the exocyclic-CH$_2$OH-group at the anomeric site of the monosaccharide. Hence, the conformational change of the fructopyranose is likely associated with a noticeably stronger rearrangement of the hydration shell, and thus more pronounced volume effect, than that of the other carbohydrates exhibiting a $\alpha C = \gamma C$ ring inversion.

The small interaction enthalpy change $\Delta U(<RT)$ required to enable chair–chair conformational isomerization is correlated with a balanced number of axial ($N_a$) and equatorial ($N_e$) exocyclic hydroxy and -CH$_2$OH groups of the monosaccharide. With $\beta$-D-arabinose, $\alpha$-D-lyxose, and $\alpha$-D-ribose, for example, $N_a/N_e = 2:2$. Since the ring inversion converts an axial group into an equatorial one, and vice versa, the $\alpha C = \gamma C$ conformational isomerization does not alter the $N_a/N_e$ ratio of those aldopentoses. An exception is

Table VIII. Anomers of the pyranose form of monosaccharides, ring conformers in water (calculated), as well as interaction enthalpy difference $\Delta U$, stoichiometric factor $\Gamma$, and volume change $\Delta V_s$ of the ring inversion.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Anomer</th>
<th>Rel. anomer</th>
<th>Ring conformers (Ref. 28)</th>
<th>$\Delta U$ (Refs. 11, 28)</th>
<th>$\Gamma$</th>
<th>$\Delta V_s$ (ml/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-arabinose</td>
<td>$\alpha$</td>
<td>0.60</td>
<td>$^1C_4$, $^4C_4$</td>
<td>4.8</td>
<td>0.08</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>0.355</td>
<td>$^1C_4$, $^4C_4$</td>
<td>2.1</td>
<td>0.15</td>
<td>1.4</td>
</tr>
<tr>
<td>D-lyxose</td>
<td>$\alpha$</td>
<td>0.70</td>
<td>$^4C_4$, $^4C_4$</td>
<td>2.3</td>
<td>0.057</td>
<td>1.4$^a$</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>0.28</td>
<td>$^4C_4$</td>
<td>0.4</td>
<td>0.115</td>
<td></td>
</tr>
<tr>
<td>D-ribose</td>
<td>$\alpha$</td>
<td>0.215</td>
<td>$^4C_4$, $^4C_4$</td>
<td>0.4</td>
<td>0.004</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>0.585</td>
<td>$^4C_4$, $^4C_4$</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-fructose</td>
<td>$\alpha$</td>
<td>0.02</td>
<td>$^2C_5$, $^2C_5$</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>0.70</td>
<td>$^2C_5$, $^2C_5$</td>
<td>11.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Mean of the volume change of both anomers.
\( \beta \)-D-ribose with its \( N_a:N_e \) ratio equal to 3:1, showing that the balanced number of axial and equatorial groups is not the only factor determining the interaction enthalpy difference. Other properties, like the arrangement of the axial and equatorial groups relative to one another, may be also important.

It is generally accepted now that cyclic molecules may adopt further conformations, in addition to the \( \alpha \) and \( \beta \) chairs. These conformations, showing up by additional potential energy minima in the Cremer–Pople space include the boat (B) and the skewed boat (S) forms. The \( \beta \)- and \( \gamma \)-relaxation terms in the acoustical spectra of saccharide solutions have been assigned to equilibria involving these less stable conformers.\(^{26}\) Interestingly, solutions of D-glucose, with its unbalanced number of axial and equatorial groups (\( \beta \)-pyranose, \( N_a:N_e = 0:5 \); \( \alpha \)-pyranose, \( N_a:N_e = 1:4 \)), and of methyl-\( \beta \)-D-glucopyranoside neither show an \( \alpha \) term nor a \( \beta \) or \( \gamma \) term in their acoustical spectra (Tables IV, VII, Ref. 25). D-xylose (\( \beta \)-pyranose, \( N_a:N_e = 0:4 \); \( \alpha \)-pyranose, \( N_a:N_e = 1:3 \)), methyl-\( \beta \)-D-xylopyranoside, and D-sorbose reveal a \( \beta \) relaxation, indicating that these molecules are capable of modes of pseudorotation but not of a complete ring inversion. D-galactose and D-mannose, the \( \alpha \)-pyranose anomers of which are characterized by \( N_a:N_e = 2:3 \), obviously form two labile ring conformers in water (Table IV). The aldopentoses \( \beta \)-D-arabinose, \( \alpha \)-D-lyxose, and D-ribose exhibit an \( \alpha \) - and a \( \beta \)-relaxation term as well (Table III). The ring inversion of these monosaccharides seems to include intermediate steps like the boat or skewed boat conformers, thus the coupled reaction scheme \( ^1 \gamma C_2 = B, S = ^4 \gamma C_1 \) appears to be appropriate to represent the conformer equilibrium. This view may, at least in parts, also account for the finding that the volume change \( \Delta V \), associated with the ring inversion, is distinctly smaller with the aldopentose than with the D-fructose solutions. With D-fructose the \( \Delta V \) values include the complete chair–chair transition, whereas with the aldopentoses \( \Delta V \) denotes the volume change from the chair to the labile intermediate (B or S) form only. The second step of the coupled reaction, the fast transition, from the intermediate to the other chair conformer, is represented by the \( \beta \)-relaxation term, the amplitude \( A_\beta \) of which reflects the volume change of this partial inversion.

We only mention the strong \( \gamma \)-relaxation term in the spectra for D-fructose in ethanol/water mixtures (Fig. 7, Ref. 44). In that solvent, the pseudorotation of fructose molecules seems to play a significant role, probably including conformational changes of the furanose tautomers, the content of which is much higher\(^ {15} \) in the mixture (0.62, \( x_e = 0.81 \)) than in water (0.28, \( x_e = 0 \)).

Recently, the \( \delta \) and \( \varepsilon \) terms in the acoustical spectra of aqueous solutions of various monosaccharides have been assigned to the rotational isomerization of exocyclic-\( \text{CH}_2\text{OH} \) side groups\(^ {26}\) and to some kind of molecular association mechanism,\(^ {26}\) respectively, including noncritical concentration fluctuations.\(^ {47\text{-}52}\) The former assignment is based on an idea of Tvaráška and Bleha\(^ {53}\) and is an obvious assumption here because the \( \delta \) term is only found for carbohydrates possessing an exocyclic-\( \text{CH}_2\text{OH} \) group.\(^ {25,26}\) Additionally, the relaxation rate \( \tau^{-1} \) is independent of the carbohydrate concentration, thus pointing at a unimolecular chemical equilibrium. The assumption of an exocyclic side group rotation is also supported by former ultrasonic relaxation\(^ {24}\) and NMR\(^ {34,55}\) studies and it is in conformity with energy differences resulting from computer models.\(^ {55}\) The latter assignment proceeds from the finding of a concentration-dependent relaxation rate \( \tau^{-1} \) of D-glucose and methyl-\( \beta \)-D-xylopyranoside.
solutions. To some extent the assumption of carbohydrate association is plausible, because of effects of concentration fluctuations that have emerged in the acoustical spectra of various aqueous solutions of organic ions and molecules, particularly of cyclic purine bases.

Within the limits of experimental error, the relaxation time $\tau_\delta$ of the fructose solutions is also independent of the saccharide concentration $c$ (Table V) and the relaxation amplitude $A_\delta$ is proportional to $c$ (Fig. 9). Within the rather small temperature range of measurements, the $\ln(\tau_\delta^{-1}/T)$ data may be represented by a linear dependence upon $1/T$, with its slope almost identical with that for D-glucose solutions (Fig. 10). These results suggest the $\delta$ term in the D-fructose solution spectra to also reflect the exocyclic-CH$_2$OH-group rotational isomerization.

The smaller slope of the $A_\delta$ versus $c$ relation for the fructose solutions (Fig. 9) may be taken as an indication of the $\delta$ term to be due to the pyranose anomers, the content of which is 72% with the D-fructose and 100% with the D(+) glucose solutions (Table I). Correspondingly, the $\epsilon$ term for the fructose system, the relaxation time $\tau_\epsilon$ of which seems to be independent of $c$ (Table V), may at least in parts be due to the exocyclic-CH$_2$OH group rotational isomerization of the fructofuranose anomers. However, the $\epsilon$ term, located between the $\delta$ term and the high-frequency asymptotic $B
u$ term, is difficult to extract from the present spectra. Additional systematic measurements, preferably including even higher frequencies, are required in order to fully account for the high-frequency $\epsilon$-relaxation term in the monosaccharide solution spectra.

**CONCLUSIONS**

Acoustical absorption spectra of monosaccharide solutions provide valuable insights in the elementary kinetics of carbohydrates in water. Depending on specific monosaccharide molecular properties, the chair–chair ring inversion, two modes of pseudorotation, exocyclic side group rotations, and, possibly a monosaccharide association mechanism dominate the molecular dynamics within the time domain between 100 ps and 20 $\mu$s (25°C).

The $\epsilon \tau_\epsilon = \epsilon \tau_\epsilon$ ring inversion, which, because of its relevance in stereochemistry and biology, is the focus of interest, has been well established now by careful investigation of the acoustical relaxation process with relaxation time $\tau_\epsilon$ around 1 $\mu$s, corresponding with a relaxation frequency $(2\pi\tau_\epsilon)^{-1} = 160$ kHz. Interaction enthalpy differences of both chair conformers in water, relaxation parameter variations with concentration and temperature, time-resolved acoustical absorption behavior of nonequilibrium tautomer systems, as well as specific characteristics in the acoustical spectra of solutions of D-fructose in ethanol–water mixtures strongly support the idea of a chair–chair conformational isomerization and of its indication by a low-frequency relaxation process. This acoustical relaxation process offers a tool to systematically investigate the significance of the carbohydrate conformational flexibility in elementary chemical reactions, including the complexation with cations, and cell recognition mechanisms.

The modes of pseudorotation of the ring structures likely reflect the existence of short-lived ring conformers, like boat and the skewed boat forms. The equilibrium between these structures and the more stable chair conformers also manifest themselves by relaxation processes, which, however, still have to be studied more systematically, particularly by including specially selected (but rather expensive) monosaccharides. The fast exocyclic-CH$_2$OH-group rotation, reflected by a relaxation with relaxation time $\tau_\delta$ at around 1 ns, is also well established now for aldohexoses and their methylated forms as well as for ketohexoses. The suggested association mechanism of some monosaccharides is characterized by an even smaller relaxation time $\tau_\epsilon$, on the order of 0.1 ns (25°C). Due to the structural variety of carbohydrates, this mechanism offers favorable conditions for the study of local fluctuations in the concentration in aqueous solutions and of their dependence upon steric properties of the solute molecules. Such noncritical concentration fluctuations have become evident recently for various organic solute water systems. Reliable investigations of the high-frequency relaxation, however, require measurements at frequencies above...
the relaxation frequency \((2\pi \tau_e)^{-1} = 1.6\) GHz. Hence, the measuring range should first be extended toward even higher frequencies.

**ACKNOWLEDGMENTS**

The authors are indebted to R. Behrends and R. Hagen for their spirited discussions. Financial assistance by the Deutsche Forschungsgemeinschaft (Bonn, Federal Republic of Germany) is gratefully acknowledged.

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