

Molecular Mobility of Sucrose in Aqueous Solution Studied by ^{13}C NMR Relaxation

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Z. Naturforsch. **48c**, 407–413 (1993); received February 24, 1993

NMR, Sucrose, Aqueous Solution, Glass Transition

From the temperature and concentration dependence of the ^{13}C spin lattice relaxation times of the 12 carbon atoms of the sucrose molecule the rotational dynamics of the sugar molecules are determined. No indication for conformational mobility of the rings is found. The exocyclic hydroxymethyl groups possess extra mobility. The models used for the description are critically discussed. The temperature dependence of the rotational mobility is described by a VTF equation. The concentration dependence of the ideal glass transition temperature T_0 for this mobility is derived.

Introduction

Concentrated aqueous solutions of saccharides are encountered rather frequently in nature. In plants and lower animals they provide nutrient depots or protection against freeze damage or anhydrobiosis. Also for many technical and pharmaceutical productions the handling of these solutions is necessary and the knowledge of their physicochemical properties is a prerequisite for process optimization. Even the macroscopic properties of the aqueous solutions as for instance the viscosity or behaviour on supercooling of mono- and oligosaccharides depend at comparable concentration critically on the stereochemistry of the dissolved carbohydrate [1].

It appears thus of interest to study the molecular dynamics of some hexose disaccharides in aqueous solution, and to learn if possible about conformational transitions of the dissolved sugar molecules and their molecular dynamics. The determination of the individual ^{13}C spin lattice relaxation times of all 12 chemical shift separated carbons of sucrose as a function of concentration and temperature is a first step towards this aim. In subsequent studies it will be attempted to apply the methods and forms of analysis described here for the most abundant disaccharide to a few selected mono- and disaccharides in order to learn which molecular features of the dissolved sugar molecules are re-

sponsible for the large differences observed in the macroscopic dynamic properties.

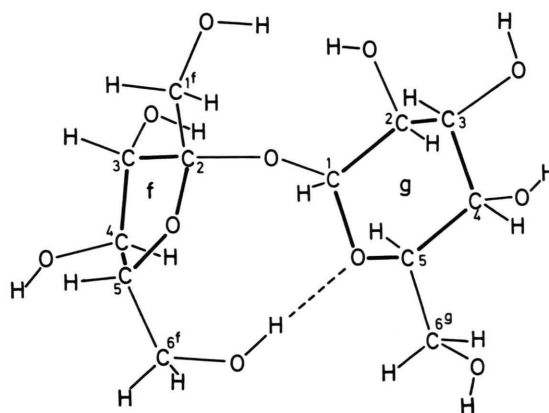


Fig. 1.

The diagram (Fig. 1) of the sucrose molecule given above shows the nomenclature used for the description of the relaxation rates of the individual carbon atoms.

The data presented here extend the temperature and concentration range of previous studies considerably [2–4].

Experimental

Sucrose (E. Merck, Darmstadt, F.R.G.) was deuterated at the hydroxyl groups by dissolving the sugar in an equal amount of D_2O (99.75% deuterated, E. Merck, Darmstadt, F.R.G.) and freeze

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Verlag der Zeitschrift für Naturforschung,
D-W-7400 Tübingen
0939–5075/93/0500–0407 \$ 01.30/0



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drying. The process was repeated three times before the final solutions were prepared. For the reliable determination of the ^{13}C spin lattice relaxation times paramagnetic oxygen has to be removed from the solutions. This was achieved by five freeze-pump-thaw cycles at a high vacuum line. The degassed samples were contained in flame sealed 10 mm NMR tubes and stored at 277 K. The ^{13}C spin lattice relaxation times were controlled repeatedly and were found to be constant for the total time span of more than one year during which the experiments were performed.

Bruker MSL 100 and MSL 300 spectrometer operating for carbon-13 at 25.09 MHz and 75.5 MHz respectively were used. The ^{13}C spin lattice relaxation times were measured with the non-selective inversion recovery pulse sequence under broad band decoupling of the protons. For the determination of the steady state nuclear Overhauser enhancement factor (NOE factor) the gated decoupling technique was applied. Home built 10 mm probe heads were used for the measurements. They are designed to minimize temperature gradients over the sample volume.

The temperature was regulated by a flow of cold or hot nitrogen with the commercial Bruker variable temperature unit. It was controlled to an accuracy of ± 0.5 K by a metal sheathed Chromel/Alumel thermocouple, which could be brought into close contact with the liquid samples. All T_1 and NOE values given are averages of at least four measurements. The individual measurements were reproducible to $\pm 3\%$.

Theory

Non-quaternary carbon-13 nuclei in diamagnetic organic molecules relax in solution predominantly by magnetic dipole-dipole interaction with the directly attached protons. This interaction is in a static magnetic field modulated by molecular rotation, thus providing the fluctuating magnetic field necessary for relaxation. The spin lattice relaxation time T_1 is given by [5]:

$$T_1^{-1} = \frac{1}{10} (\gamma_H \gamma_C \hbar)^2 \sum_H \langle r_{CH}^{-3} \rangle^2 \{ J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C) \} \quad (1)$$

with γ the gyromagnetic ratio and $J(\omega_d)$ the spectral density function at the resonance frequency of nucleus d at the magnetic field applied.

The nuclear Overhauser enhancement (NOE) factor η *i.e.* the increase in signal intensity of the ^{13}C signal under proton broadband decoupling conditions is [6]:

$$\eta = \frac{\gamma_H}{\gamma_C} \frac{6J(\omega_H + \omega_C) - J(\omega_H - \omega_C)}{J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)} \quad (2)$$

$J(\omega)$ is the Fourier transform of the correlation function $G(\tau)$ which describes the decay of memory in a rotating molecular system:

$$J(\omega) = \int_0^\infty G(\tau) \cos(\omega\tau) d\tau \quad (3)$$

$$G(\tau) = \langle A(0)A(\tau) \rangle \quad (4)$$

with $A(\tau)$ a second order spherical harmonic, and the brackets $\langle \rangle$ representing a time correlation function [7].

All information for molecular rotational motion obtainable from a NMR experiment is contained in $J(\omega)$.

For a rigid, isotropically tumbling molecule the correlation function can be described by a single exponential:

$$G(\tau) = e^{-\frac{|\tau|}{\tau_c}} = e^{-D\tau} \quad (5)$$

where τ_c and D are the correlation time and the rotational diffusion constant, respectively.

The Fourier transformation of Eqn. (5) yields the spectral density:

$$J(\omega) = \langle A^2(0) \rangle \frac{\tau_c}{1 + (\omega\tau_c)^2} \quad (6)$$

The initial amplitude factor ($1 < \langle A^2(0) \rangle > 0$) takes the contribution from internal motion into account. A rapid initial decay in $G(\tau)$ caused by angular components of local librations. However, the librations alone are incapable of averaging an intermolecular vector over all possible orientations. The correlation function reaches a plateau value very rapidly ($\approx ps$) from which it decays by overall molecular tumbling with the rotational correlation time τ_c . The effect of the rapid libration is thus the reduction of the amplitude of $J(\omega)$ at NMR frequencies. The amplitude factor decreases with the increase of librations.

The relaxation behaviour for the higher concentrated solutions cannot be expressed in terms of an exponential relaxation process with a single correlation time. It is quantitatively described by a superposition of exponential relaxation processes,

which then lead to a distribution of correlation times $g(\tau)$ that may be represented by a Cole-Davidson-equation [8]:

$$G(\tau) = \int_0^\infty e^{-\frac{t}{\tau}} g(\tau) d\tau \quad (7)$$

$$g(\tau) = \begin{cases} \frac{\sin(\beta\tau)}{\pi} \left(\frac{\pi}{\tau_0 - \tau} \right)^\beta & 0 < \tau < \tau_0 \\ 0 & \tau > \tau_0 \end{cases} \quad (8)$$

$$J(\omega) = \frac{\sin(\beta \arctan(\omega\tau_0))}{\omega(1 + (\omega\tau_0)^2)^{\frac{\beta}{2}}} \quad (9)$$

with β the CD-parameter characterizing the width of the distribution and τ_0 the long time limit (cut-off time).

The temperature dependence of the correlation time is well represented by the empirical VTF-equation [9]:

$$\tau_c = \tau_\infty e^{\frac{B}{(T-T_0)}} \quad (10)$$

where T_0 denotes the ideal glass transition temperature. It predicts a slowing down of cooperative molecular motions at T_0 . The experimentally determined glass transition temperature T_G is found 10–20 K above T_0 [10].

Dynamics of the exocyclic CH_2OD -groups

For a rigid isotropically tumbling sucrose molecule in solution the ^{13}C spin lattice relaxation rate of the CH_2OD -group should be twice the value of the $> \text{CHOD}$ -ring carbons ($T_1^{-1} \sim N_H$). Experimentally a value of approximately 1.7 is found. This indicates, that the hydroxymethylgroups possess an extra rotational mobility around their carbon-carbon bond. Several models given in the literature have been tested for the description of this extra mobility.

The Woessner model [11] describes the extra mobility as rotational jump diffusion of the C–H-vector around a rigid C–C axis a fixed angle ϑ between the axis of rotation and the C–H bond is assumed while the rotational jumps occur in 120° steps.

$$J(\omega) \xrightarrow{\tau_i \rightarrow 0} \left\{ \left(\frac{3 \cos^2 \beta - 1}{2} \right)^2 + 3 \sin^2 \beta \cos^2 \beta \left(\frac{\sin \vartheta}{9} \right)^2 + \frac{3}{4} \sin^4 \beta \left(\frac{\sin 2\vartheta}{2\vartheta} \right)^2 \right\} \left\{ \frac{\tau}{1 + (2\omega\tau)^2} \right\}. \quad (15)$$

The Allerhand model [12] assumes under the same geometric constraints random small angle steps of bond rotation. Neither model is appropriate to describe the experimental data for the whole concentration range with one consistent set of parameters.

The model free approach [13] for the interpretation of $J(\omega)$ starts with the assumption of isotropic tumbling of the complete molecule but assumes no specific geometric or dynamic model for the internal motion. If the overall and internal motions are independent, the total correlation function can be factorized:

$$G(t) = G_o(t) \cdot G_i(t). \quad (11)$$

The correlation function for the overall motion is equal to Equation (5). The internal motion is given by:

$$G_i(t) = \langle P_2(\hat{\mu}(0)\hat{\mu}(t)) \rangle \quad (12)$$

where the unit vector $\hat{\mu}$ describes the orientation of the interaction vector in a frame that is rigidly attached to the sucrose molecule.

The spectral density function for the model free approach can be written as:

$$J(\omega) = S^2 \frac{\tau_c}{1 + (\omega\tau_c)^2} + (1 - S^2) \frac{\tau}{1 + (\omega\tau)^2}. \quad (13)$$

$$\tau^{-1} = \tau_c^{-1} + \tau_{\text{eff}}^{-1} \quad (14)$$

τ_{eff} is an effective correlation time for the internal motion and S is a generalized order parameter. S is a model independent measure for the degree of spatial restriction of the motion and must have a value between 0 and 1. If the internal motion is isotropic then $S = 0$, if the motion is completely restricted then $S = 1$. For concentrations higher than 50% also the model free approach cannot describe the experimental data.

All models described above are based on diffusion over a continuum or jumps between several discrete states. In the following model the range of motion possible is limited only by boundary conditions for the internal mobility. For restricted diffusion about a rigid axis an analytical solution in the limit $D_i \rightarrow \infty$ has been given [14]. The resulting spectral density function is:

Here β is the angle between the relevant C–H relaxation vector and the internal axis of rotation, 2θ defines the allowed range of motion. This model fits the experimental data over the whole concentration range.

Results and Discussion

In most experiments the spin lattice relaxation time of the individual carbons could be determined. At very low temperatures and high concentrations the short spin-spin relaxation times broaden the signals and one envelope for all 8 ring carbon carrying one directly attached proton is observed only. Even under these conditions the signal for the three hydroxymethylgroups are chemical shift separated from the other carbons and permit a determination of the spin lattice relaxation time for these carbons. In the complete c, T-range where individual T_1 values can be determined, all eight ring carbons carrying one directly bound proton yield identical spin lattice relaxation times within the precision of the experimental data. The eight C–H-bonds of these carbons which are the main relaxation vectors point into all directions of space (Fig. 1). They are thus sensitive to the rotational motion around different axes of the sucrose molecule. Their identical relaxation times prove, that the mobility of the dissolved sucrose molecule can be described by the isotropic rotational tumbling of a rigid molecule. These data also show that the ring puckering does not contribute to the ^{13}C relaxation. It is to be expected, that the furanose ring of the fructose moiety possesses a greater conformational flexibility than the sixmembered pyranose ring structure of the glucose part, thus conformational ring puckering transitions should preferably lengthen the ^{13}C spin lattice relaxation times of the five membered ring. Experimentally, however, identical T_1 -values are found in both moieties.

In Fig. 2 the spin lattice relaxation time of the eight carbons are given at two magnetic fields. For all higher concentrations they pass through the minimum of T_1 predicted by relaxation theory for sufficiently long rotational correlation times. At constant magnetic field the minimum is independent of concentration. From the minimum condition the dipolar coupling constant and thus the C–H bond length can be derived (Eqn. (1)). At

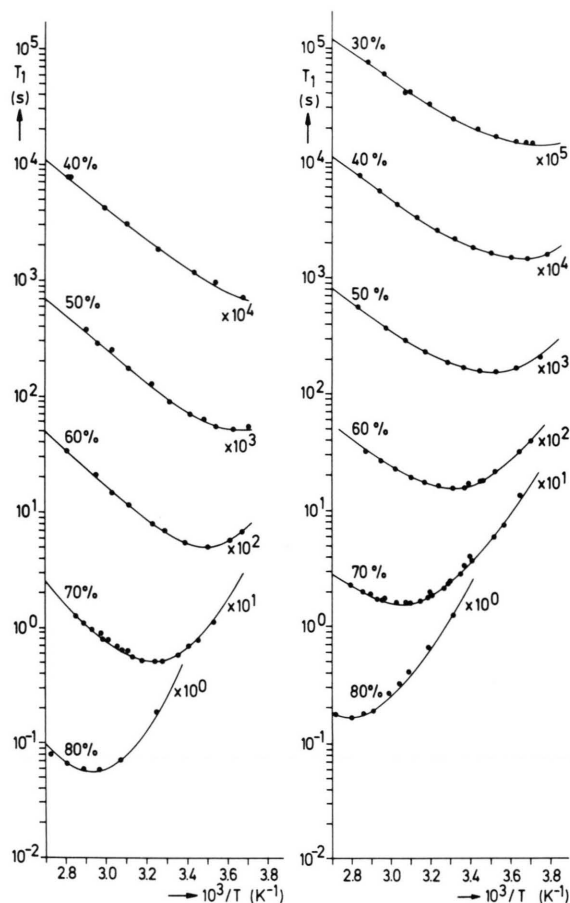


Fig. 2. Temperature dependence of the ^{13}C spin lattice relaxation time T_1 of the tertiary ring carbons of sucrose at various concentrations. Lines drawn through the experimental points result from fitting of the data to Eqn. (1). Concentrations given are % w/w. Left side: 2.35 Tesla, right side: 7.05 Tesla.

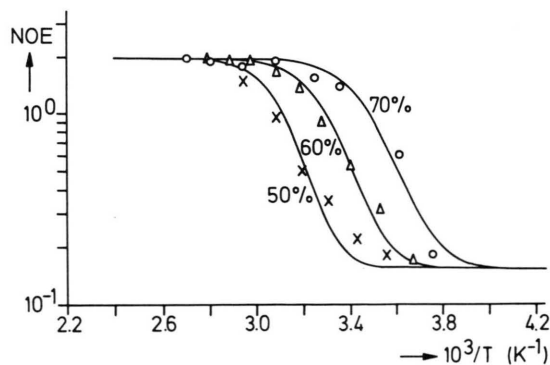


Fig. 3. NOE enhancements of the ^{13}C signals of the eight tertiary ring carbons of sucrose as function of temperature for three concentrations at 2.35 Tesla. Concentrations given are % w/w.

Table I. Fit parameter derived from ^{13}C spin lattice relaxation times T_1 for the ring carbons carrying one proton.

w/w [%]	B [K]	T_0 [K]	DCC [kHz]	τ_0 [s]	$\langle A^2(0) \rangle$	β
30	1060	134	136.52	5.6×10^{-13}	0.95	1
40	1060	140	136.52	5.6×10^{-13}	0.90	1
50	1060	152	136.52	7.1×10^{-13}	1	0.70
60	1060	168	138.52	8.5×10^{-13}	1	0.64
70	1060	192	146.55	11.6×10^{-13}	1	0.50
80	1060	222	146.65	11.6×10^{-13}	1	0.44
90	1060	248	146.65	12.6×10^{-13}	1	0.42

higher temperature the data at both frequencies at all concentrations and also the NOE enhancement factor η (Fig. 3) can be described by the motion of an isotropically rotating molecule characterized by a single correlation time τ_c . The lines drawn through the experimental points in Fig. 2 and 3 result from fitting the experimental data to Eqns. (1) and (2) assuming a VTF-temperature dependence (Eqn. (10)) of the single relaxation time. The parameter of these fits are compiled in Table I. The dipolar coupling constant derived from these fits leads to an effective bond length $\langle r_{\text{CH}} \rangle = 1.124 \text{ \AA}$. Protons on neighbouring molecules could contribute approx. 5% to these relaxation as simple inspection of threedimensional models shows, thus the actual bond length of the ring C–H structures should become even larger, compared to this the static C–H bond length determined by scattering experiments is $r_{\text{CH}} = 1.11\text{--}1.12 \text{ \AA}$ [15, 16]. This shows unambiguously that librational motions lead to an effective reduction of the dipolar coupling. Introducing for a $\langle A^2(0) \rangle$ a value of 0.95 and 0.90 into Eqn. (6) leads to a more reasonable static r_{CH} .

For $c(\text{sucrose}) \geq 50\%$ w/w the T_1 -curves show a wider and unsymmetric minimum. In order to fit the experimental T_1 to these curves a Cole-Davidson distribution of correlation times (Eqn. (9)) has to be introduced. The parameter of this distribution are also given in Table I. It is reasonable to assume that in the highly concentrated solutions, where diffusion is slowed down, the individual sucrose molecules can rest in different local environments *i.e.* in different local sugar and water concentrations for time spans larger than T_1 and the distribution of correlation times would be a natural consequence of this incomplete averaging out of the molecular solvation spheres.

On the high temperature side the spin lattice relaxation times of the ring carbons at all concentrations can be projected onto a single curve, by plotting them on a modified Arrhenius diagram, where the absolute temperature is replaced by the difference between the actual temperature and the glass transition temperature $T_0(c)$. The latter being obviously the only concentration-dependent quantity. This reduction is given in Fig. 4 for the measurements at 7.05 T and is valid for the temperatures $T > T_0 + 130 \text{ K}$, with the concentration dependent T_0 as given in Table I. Fig. 6 shows that the concentration dependence of T_0 as obtained from the fits parallels exactly the experimental T_G as derived from calorimetric studies [17, 18]. T_G is found approx. 10 K higher than T_0 . Thus the ideal glass transition temperature is not really a free fit parameter but is corroborated by other experimental studies (Fig. 5).

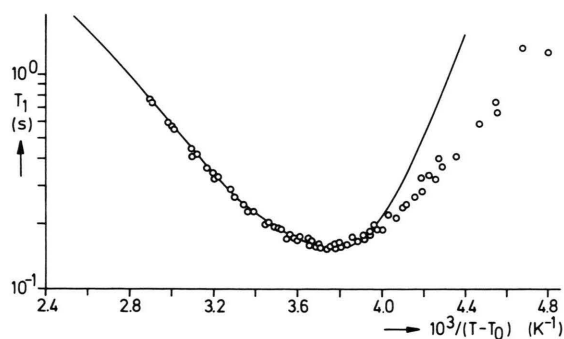


Fig. 4. ^{13}C spin lattice relaxation times T_1 at the eight tertiary ring carbons of sucrose in a modified Arrhenius. Plotted as $(T - T_0)$ with T_0 the ideal glass transition temperature as obtained from the fits with the parameter collected in Table I.

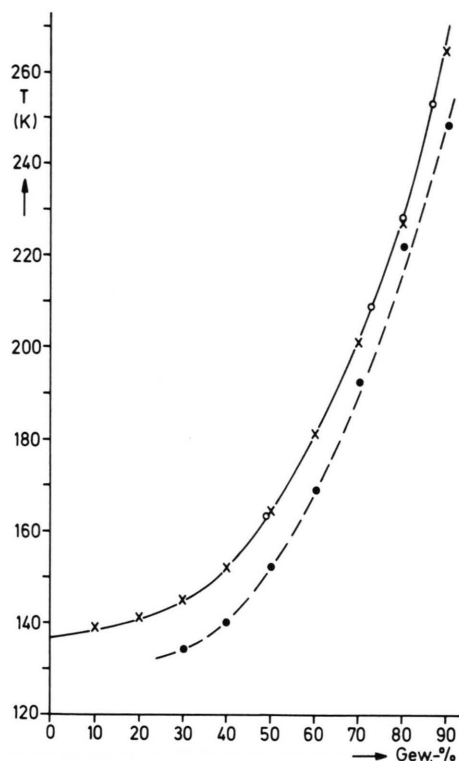


Fig. 5. Comparison of the ideal glass transition temperatures T_g obtained for the ring rotational mobility with the experimental calorimetric T_g [18, 19].

Relaxation of the hydroxymethyl groups

In a rigid isotropically tumbling sucrose molecule the exocyclic hydroxymethyl groups should relax twice as fast as the ring C–H carbons because of the two protons bound to them. Experimentally the minimum in the T_1 -curves (Fig. 6) is only a factor

$$\text{1.54–1.74 lower } \left(\frac{T_1(\text{CH})}{T_1(\text{CH}_2\text{OD})} = 1.54\text{--}1.74 \right).$$

Thus rotational mobility around the exocyclic C–C bond must contribute to the ^{13}C relaxation of these groups.

At constant concentration the temperature dependence of the ^{13}C - T_1 is identical for the C-1^f and C-6^g group, while for the C-6^f group a significantly different temperature dependence and a higher mobility is observed. This is ascribed to the formation of an intramolecular hydrogen bond between the C-1^f hydroxyl and sterical hindrance in the case of the C-6^g group [19].

For this extra mobility neither the Woessner nor the Allerhand model permits the fitting of the T_1 data over the whole c,T-range. The fit to these models is only possible at $c \leq 50\%$ w/w. All data can however be described by the restricted diffusion model given in Eqn. (15). The temperature dependence of the rotatory motion around the C–C

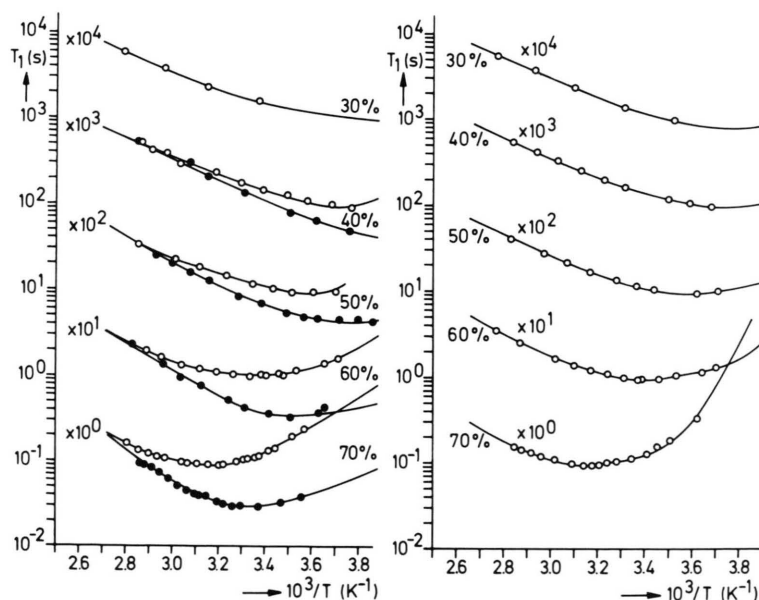


Fig. 6. Temperature dependence of the ^{13}C spin lattice relaxation rates of the exocyclic hydroxymethyl groups at various concentrations. Results for the C-6^f and C-1^f group are on the left side. Right side C-6^g group. Full circles 2.35 Tesla, open circles 7.05 Tesla. Lines drawn through the experimental points result from fitting the data to Eqn. (15) with the parameter compiled in Table II.

bond is found to be Arrhenian expect for the C-6^f group in the most concentrated solutions. The activation energies derived are identical within experimental error. All fit parameter are compiled in Table II.

In Fig. 7 the ratio of the correlation times $\frac{\tau(\text{ring})}{\tau(\text{hydroxymethyl})}$ are plotted for the C-6^g and C-1^f group at 300 K. It is obvious from this graph, that the increase of $\tau(\text{ring})$ with concentration is not accompanied by a corresponding increase in $\tau(\text{hydroxymethyl})$. The extra mobility of this group is entirely intramolecular and thus fairly independent of solvent viscosity.

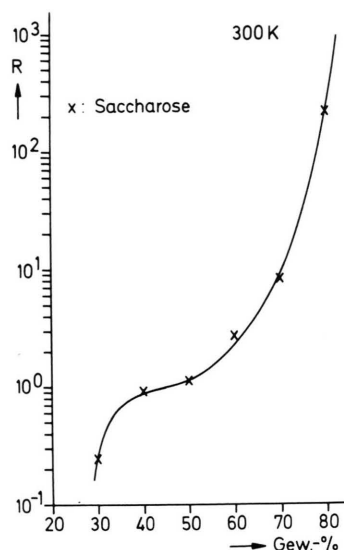
Acknowledgements

Financial support bei the Deutsche Forschungsgemeinschaft is gratefully acknowledged. D. Girlich thanks the Friedrich-Ebert-Stiftung for a fellowship.

Fig. 7. Ratio $R = \frac{T_1(\text{ring})}{T_1(\text{hydroxymethyl})}$ of the rotational correlation times of the ring and exocyclic hydroxymethyl carbons as obtained from the fits of the relaxation data with the parameter given in Tables I and II.

Table II. Fit parameter from ^{13}C spin lattice relaxation times for the hydroxymethyl groups C-1^f, C-6^g (restricted diffusion Eqn. (15)).

w/w	30%	40%	50%	60%	70%	80%
E_A $\left[\frac{\text{kcal}}{\text{mol}} \right]$	4.96	4.30	4.65	4.82	5.32	5.56
τ	22	26	26	26	26	26



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