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# Ultrasonic and dielectric study of nonequilibrium monosaccharide solutions in water

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**Abstract**

Based on broadband acoustical (10 kHz ≤ $\nu$ ≤ 2 GHz) and dielectric (1 MHz ≤ $\nu$ ≤ 40 GHz) spectrometry, time-resolved ultrasonic attenuation coefficient and static permittivity measurements have been performed on nonequilibrium tautomer solutions of D-arabinose and D-fructose in water. Via the chair–chair ring inversion the ultrasonic attenuation measurements display the decrease in the content of β-arabinopyranoside and the increase of the α-fructopyranoside concentration during the establishment of the tautomer equilibrium. For the arabinose solutions, the mutarotation decay constant ($m$=(0.027 ± 0.004) min$^{-1}$, 20 °C) from the ultrasonic measurements almost agrees with that from optical activity observations. For D-fructose the ultrasonic decay constant ($m$=(0.043 ± 0.007) min$^{-1}$, 20 °C) is smaller than that from rotary polarization ($m$ = 0.054 min$^{-1}$, 20 °C) and dielectric permittivity ($m$=(0.058 ± 0.007) min$^{-1}$, 20 °C), likely because the latter methods probe parallel pathways in the tautomer equilibrium whereas the former one reflects only one pathway.


*Keywords:* Mutarotation; Ring inversion; Saccharide solutions; Ultrasonic attenuation coefficient; Dielectric permittivity

## 1. Introduction

Constituting the most abundant class of material in the biosphere carbohydrates hold a key position in the living nature [1,2]. Considerable efforts are therefore directed towards a better understanding of the relationship between the carbohydrate structure and biological functions [3]. Particularly the broad and diverse roles of carbohydrates in processes such as protein folding, cell signaling and recognition, leukocyte trafficking, tumor cell metastasis and hormone activity regulation, as well as applications in sugar-based drugs [4] and in the osmoregulation of tissues and organs are the focus of current interest. The investigations into modern fields of glycobiology have stimulated employment of a variety of experimental techniques [5–10], including broadband ultrasonic [11–16] and dielectric [17–22] relaxation methods, to study the solution properties of carbohydrates.

In Fig. 1, ultrasonic excess attenuation-per-wavelength spectra for 1 molar aqueous solutions of D-ribose and L-sorbose are presented. Excess attenuation

$$(\alpha\lambda)_{\mathrm{exc}} = \alpha\lambda - B\nu \tag{1}$$

is shown in order to accentuate the relaxation characteristics. In Eq. (1), $\alpha$ is the sonic attenuation coefficient at frequency $\nu$, $\lambda$ is the wavelength of the sonic field and $B$ is the frequency independent coefficient of an asymptotic high frequency background contribution, predominantly due to viscous friction. This contribution is of minor interest here. The spectra in Fig. 1 display the altogether five relaxation regimes, which have been found in the ultrasonic attenuation coefficient of monosaccharide solutions so far. The relaxation times $\tau_i$=$(2\pi\nu_i)^{-1}$, $i$ = α,. . .,ϵ are on the order of 1 μs, 100 ns, 10 ns, 1 ns and 100 ps, respectively. The relaxation terms have been assigned to a chair–chair ring inversion (α), to two modes of pseudoration ($\beta$, $\gamma$), to the exocyclic hydroxymethyl group isomerization ($\delta$) and to an association mechanism (ϵ), likely a dimerization [12,16]. As illustrated by the complex (dielectric) permittivity spectrum ($i^2 = -1$)

$$\epsilon(\nu) = \epsilon'(\nu) - i\epsilon''(\nu) \tag{2}$$

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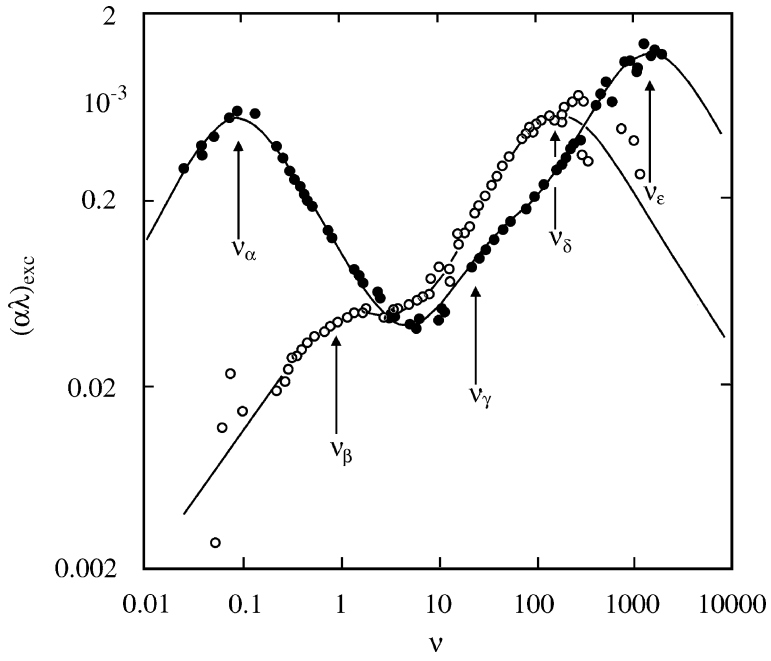


Fig. 1. Ultrasonic excess attenuation spectra for 1 mol/ℓ solutions of D-ribose (●) and L-sorbose (○) in water at 25 °C. The $\nu_i$, $i=\alpha,\ldots,\epsilon$ denote relaxation frequencies of different relaxation terms in the spectra.

of a 1 mol/ℓ solution of D-fructose in water (Fig. 2) the ultrasonic relaxation regimes are complemented by a dielectric relaxation with relaxation time $\tau=(2\pi\nu_d)^{-1}$ at around 10 ps. The dielectric relaxation reflects the hydrogen bond network fluctuations of the solutions, in close correspondence to the spectrum for water at the same temperature (Fig. 2).

Although the ultrasonic and dielectric spectra of saccharide solutions evidence the association behaviour of carbohydrates as well as the flexibility and conformational variety of the ring molecules, assumed to play a fundamental role in a biochemical alphabet beyond the genetic code [23], no information has been obtained from broadband spectrometry on the tautomer equilibrium of this class of molecules. The relaxation times characterizing the establishment of this chemical equilibrium are too large to directly show up in the spectra. Here, we combine relaxation spectrometry with time resolved ultrasonic at-

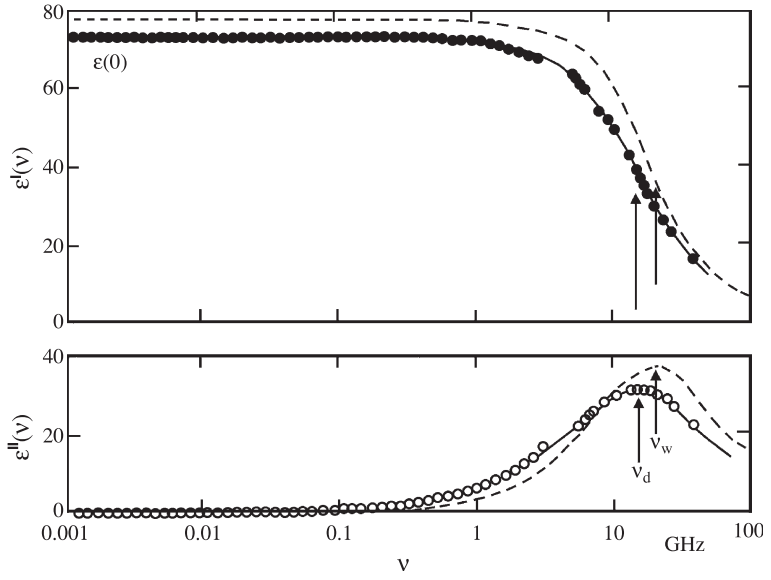


Fig. 2. Real part $\epsilon'$ and negative imaginary part $\epsilon''$ of the complex dielectric spectrum of an aqueous solution of D-fructose (1 mol/ℓ, symbols) and of water (dashed lines) at 25 °C.

Table 1
Pyranose anomer content, interaction enthalpy differences, and ring conformers of D-arabinose and D-fructose in aqueous solutions at room temperature [24,25]

| Saccharide | Anomer | Rel. conc. (%) | Interaction enthalpy difference (kJ/mol) | Ring conformers |
|---|---|---|---|---|
| D-Arabinose | α | 60.0 | 4.8 | $^1C_4$ |
| | β | 35.5 | 2.1 | $^1C_4$, $^4C_1$ |
| D-Fructose | α | 2.0 | 2.7 | $^2C_5$, $^5C_2$ |
| | β | 70.0 | 11.5 | $^2C_5$ |

tenuation and complex permittivity measurements to provide a method of determining the relaxation times in the coupled scheme of tautomer equilibria.

## 2. Experimental

The broadband ultrasonic [12,16] and dielectric [20,22] spectra of monosaccharide solutions are described elsewhere. Time-resolved measurements have been performed on D(−)-arabinose and D(−)-fructose solutions at various concentrations and temperatures. Both monosaccharides have been purchased as β-pyranose anomers from SIGMA (Deisenhofen, Germany, ≥99%) and have been used as delivered. Solutions were prepared by weighing the saccharides into suitable flasks, which afterwards have been filled up to the fiduciary mark using prethermostated water. After stirring, the samples were degassed and immediately filled into the thermostated cells. Measurements started at the last 10 min after addition of water to the saccharide.

The time-resolved sonic attenuation coefficient measurements proceed from the idea that the ${}^iC_j \rightleftharpoons {}^jC_i$ ring inversion couples to the β-pyranose anomer of arabinose and to the α-pyranose anomer of fructose but not to the complementary pyranose anomers. As indicated by the interaction enthalpy differences for the chair-conformers listed in Table 1, the activation energies for the ring inversion of α-D-arabinopyranose and β-D-fructopyranose are significantly larger than thermal energy RT ≈ 2.5 kJ/mol at room temperature. Hence, a chair−chair ring conformational change of these latter anomers is unlikely. The amplitude $A_\alpha$ in the α-relaxation term $2\pi\nu\tau_\alpha A_\alpha\,(1+4\pi^2\nu^2\tau_\alpha^2)$ of D-arabinose (Fig. 1) and D-fructose (Fig. 3) solutions is thus proportional to the concentration of the β- and α-pyranose anomer, respectively. In addition, the relaxation time for a unimolecular reaction does not depend on concentration. Such behaviour has been verified by broadband spectrometry of solutions with different saccharide content (Fig. 3). Hence, at $\nu < 2$ MHz, the amplitude $A_\alpha$, and thus the concentration of the anomer species that is capable of the ${}^iC_j \rightleftharpoons {}^jC_i$ ring inversion, is proportional to the $(\alpha\lambda)_{exc}$ value at a suitable frequency $\nu_{meas}$. We have measured the attenuation-per-wavelength utilizing a plano-concave cavity resonator filled with the solution [26]. In this kind of measurements, in a first approach, $\alpha\lambda$ at the measuring frequency is proportional to the half-power bandwidth of the resonance curve with resonance frequency $\nu_{meas}$. We used a computer-controlled network analyzer to scan the resonance curve in a frequency range around $\nu_{meas} = 380$ kHz, where the intrinsic cell losses adopted their minimum value. Each scan required 3.6 s so that we were able to determine the half-power bandwidth every 11 s.

Another network analyzer has been used to measure in the frequency range between 1 MHz and 3 GHz the electrical input impedance of an easy-to-handle cell from the cut-off variety [27] as a function of time.

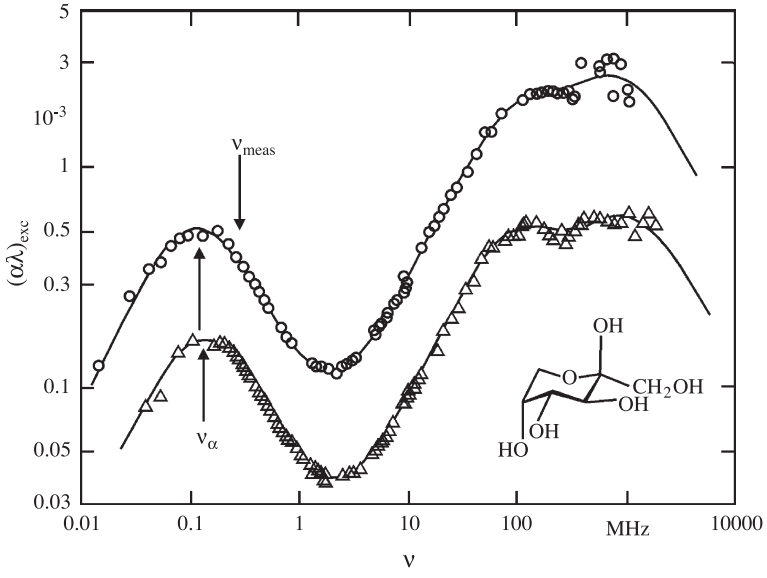


Fig. 3. Ultrasonic excess attenuation spectra for aqueous solutions of D-fructose at 25 °C with saccharide concentrations 0.5 mol/ℓ (Δ) and 1.5 mol/ℓ (○). $\nu_{meas}$ indicates the frequency of measurement in the time-resolved measurements.

At $\nu < 500$ MHz, the real part $\epsilon'$ of the dielectric spectrum of the solutions under consideration is independent of frequency (Fig. 2). Hence, the static permittivity $\epsilon(0) = \lim_{\nu \to 0} \epsilon'(\nu)$ of the samples followed directly from the measurements.

The time dependence of the optical activity of freshly prepared solutions was obtained from rotatory polarization measurements at $\lambda = 485$ nm using a 100-mm cell positioned between suitable polarizers.

## 3. Results and discussion

From polarimetry, $^{13}$C-NMR measurements and gas–liquid chromatography several authors concluded that, in aqueous D-fructose solutions at room temperature, only traces of the α-pyranose tautomer exist at the most. A compilation of the tautomer composition of solutions of D-fructose in water, as following from different methods, is given by Flood et al. [28]. Nonexistence of α-D-fructopyranose in aqueous solutions would mean that the α-relaxation term in the ultrasonic spectra of D-fructose (Fig. 3) cannot be due to the chair–chair ring inversion. The time-resolved nonequilibrium measurements of freshly prepared D-fructose solutions, however, strongly support the assignment of the low-frequency ultrasonic relaxation to the conformational equilibrium of the α-pyranose tautomer. In Fig. 4, the time development of

$$F(t) = (Y(t) - Y(\infty))/\Delta \tag{3}$$

is shown as a function of time $t - t_o$ after start of the measurements at $t_o$. In the following, $Y$ may denote anyone of the experimental quantities under consideration. In Fig. 4, it equals the half-power bandwidth of the resonator at 380 kHz, corrected for the effect of intrinsic losses. In Eq. (3), $\Delta = |Y(t_o) - Y(\infty)|$. With the D-fructose solution, according to our above arguments, $Y$ is proportional to the concentration of α-D-fructopyranose. Hence, the finding of $F(t)$ to increase with $t$ suggests the formation of the α-pyranose anomer until the tautomer equilibrium is established. The assignment of the α-relaxation to the chair–chair conformational change, coupled to the pyranose anomer equilibrium, is additionally supported by the $F(t)$ function for the arabinose solution. With this sample the content of the β-pyranose tautomer, which is capable of a $^1C_4 \rightleftharpoons {}^4C_1$ ring inversion, decreases during the establishment of the tautomer equilibrium (Table 1). As revealed by Fig. 4, likewise decreases $F(t)$.

For the first 20 min after the beginning of the measurements, the amount of $F(t)$ is logarithmically displayed

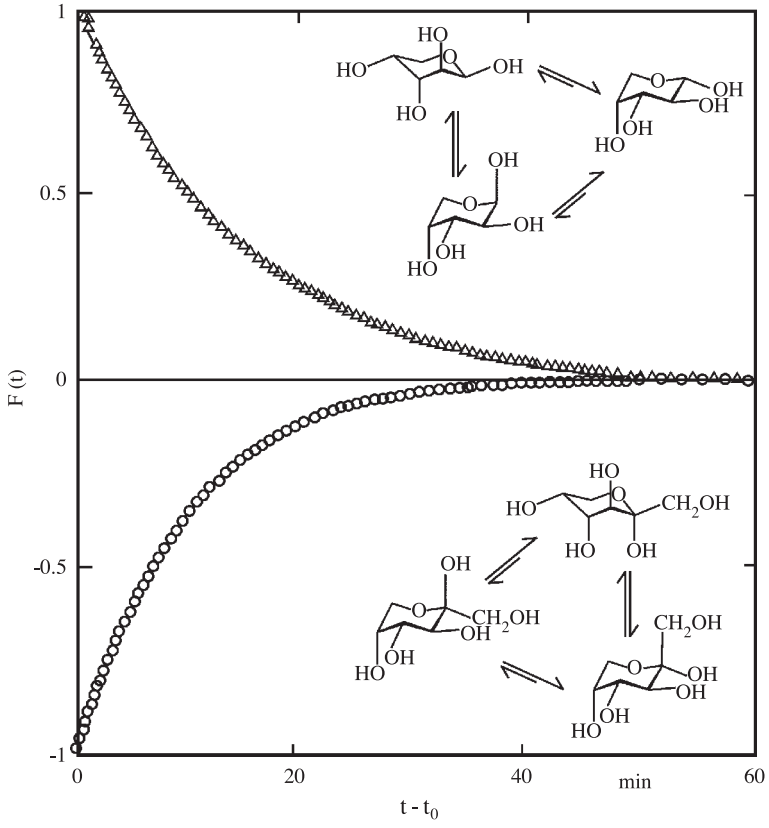


Fig. 4. Time dependence in the corrected resonator half-power bandwidth, proportional to the ultrasonic attenuation coefficient at 380 kHz, of freshly prepared aqueous solutions of D-arabinose (Δ, 1.5 mol/ℓ) and D-fructose (○, 1 mol/ℓ) at 20 °C. Measurements started at $t_o = 3$ min after preparation of the sample.

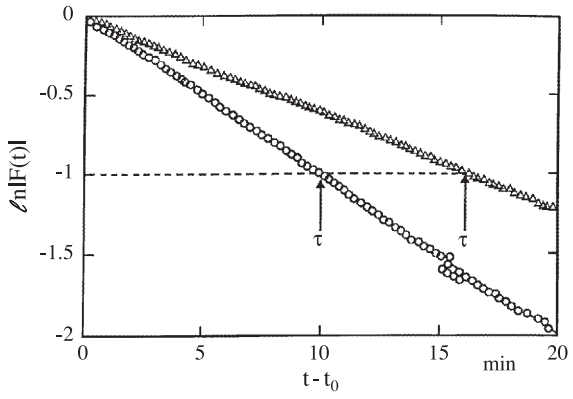


Fig. 5. Logarithmic plot of the amount of the relative half-power bandwidth for the first 20 min part of the curves displayed in Fig. 4.

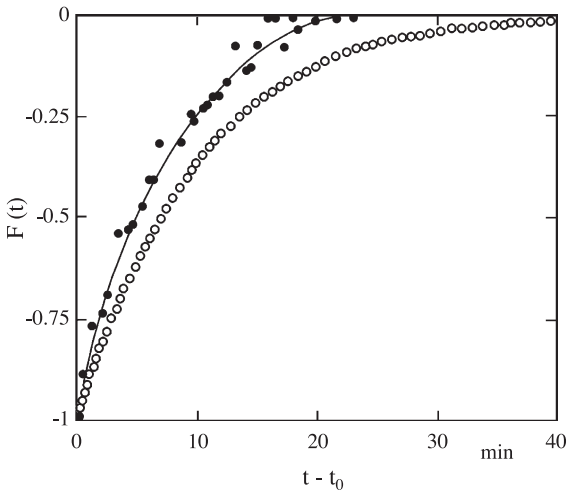


Fig. 7. Relative variation of the static permittivity (●, $Y=\epsilon(0)$) and of the ultrasonic attenuation coefficient at 380 kHz (○, $Y=\alpha$) of an 1 mol/ℓ aqueous solution of D-fructose at 20 °C.

versus $t - t_o$ in Fig. 5, in order to show how nicely the data follow an exponential:

$$|F(t)| = e^{-t/\tau} \quad (4)$$

The relaxation time $\tau$ is about 16 min for D-fructose in water at 20 °C. Following the convention of optical activity measurements of the tautomer kinetics, the time dependence in $F$ may be expressed as

$$F(t) = 10^{-mt} \quad (5)$$

where $m=(0.027 \pm 0.004)$ min$^{-1}$ for D-arabinose and $m=(0.043 \pm 0.007)$ min$^{-1}$ for D-fructose at 20 °C. The errors include contributions from an incomplete constancy of the sample temperature during the measurements immediately after filling the specimen cells. Optical activity measurements yielded $m=0.03$ min$^{-1}$ for the former and $m=0.054$ min$^{-1}$ for the latter saccharide at 20 °C. Within the limits of experimental errors, the acoustical and optical measurements yield the same decay constant for the arabinose solution for which the decrease in the β-pyranose content is probed ultrasonically. For the D-fructose solution, the acoustically determined $m$ is somewhat smaller than that from the optical activity measurement. The smaller decay constant and thus larger relaxation time may be due to the fact that in this case the acoustical measurement refers to the conversion of β-pyranose to α-pyranose only, whereas optical activity includes parallel pathways to the furanose forms (Fig. 6).

As shown by Fig. 7, the static dielectric permittivity of a nonequilibrium fructose solution also increases somewhat faster than the ultrasonic attenuation coefficient. Likely, the

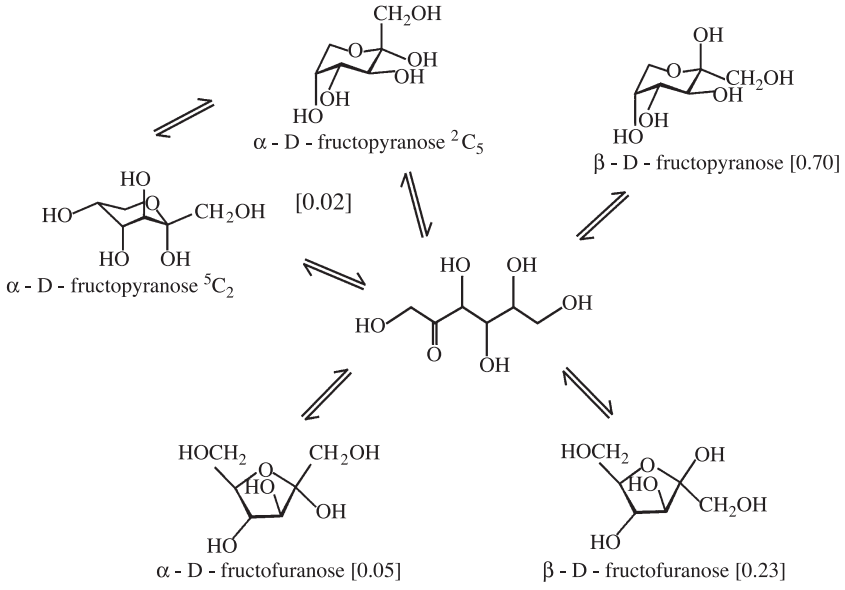


Fig. 6. Tautomer scheme of D-fructose in water. Figures in brackets indicate the relative equilibrium content of the tautomers.

permittivity change is predominantly due to the formation of the furanose anomers with a freely rotable dipolar hydroxymethyl group in excess to the pyranose rings. Again, two parallel pathways are offered by the mutarotation scheme (Fig. 6) for the formation of an exocyclic group with equilibrium content 28%, contrasting the formation of α-fructopyranose with the small equilibrium concentration of 2%.

## 4. Conclusions

The establishment of the tautomer equilibrium of D-arabinose and D-fructose from nonequilibrium concentrations in aqueous solutions can not just be followed by rotary polarization but also by ultrasonic attenuation coefficient and static (dielectric) permittivity measurements. The ultrasonic attenuation coefficient couples to the chair–chair ring inversion of the β-arabinopyranoside and the α-fructopyranoside. Starting with the β-pyranose tautomers, the time-resolved ultrasonic measurements of arabinose thus show the decreasing concentration of this species during establishment of the equilibrium, whereas they reveal the increasing content of the α-pyranose anomer in the fructose solutions. For arabinose, the mutarotation time constants derived from the ultrasonic attenuation and the rotary polarization measurements thus almost agree. For the fructose system, the relaxation time from the ultrasonic measurements is larger than those from optical activity and static permittivity observations, likely because the former technique probes only one pathway in the coupled tautomer equilibrium, whereas the others include at least two parallel pathways.

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