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Conceivably the first example of a phase transition in aqueous solutions of oligosaccharide glycosides. Evidence from variable-temperature ^{1}H NMR and optical rotation measurements for a solution of allyl lactoside

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In recent years, carbohydrates and (neo)glycoconjugates have found ever-increasing use in glycobiology and are also applied in the preparation of pharmaceuticals, artificial antigens, diagnosticums, and vaccines.[1] Very important characteristics of these compounds can be obtained by NMR spectroscopy. Measurements of ^{1}H NMR spectra of complex natural and synthetic carbohydrates at elevated temperature (313—343 K) is a routine approach to enhancing the apparent spectral resolution. We studied the influence of the temperature (in the range of 292—343 K) on the ^{1}H NMR spectral pattern (Bruker AC-200, 200.13 MHz) of a 0.2 *M* solution of allyl lactoside (allyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (**1**))[2] in D_2O. In this case, the visually observed spectral resolution was also improved with temperature. The quantitative measurements were performed for certain groups of signals. It appeared that the "widths" ($w^*_{1/2}$) of the transformed* signals for the anomeric proton (δ 4.50 at 297 K) and one of the methylene protons of the double bond of the allyl group (δ 5.25 at 297 K) observed in the spectra of **1** sharply decreased as the temperature was increased in the range of 300—303 K. At the same time, these "widths" remained virtually unchanged at higher temperatures (Fig. 1, *a*). The visually observed resolution of the remaining signals also changed jumpwise at temperatures above 303 K. However, it is difficult to quantitatively estimate this enhancement of the resolution due to overlapping of the signals. The observed jumpwise change in the apparent resolution cannot be accounted for (*cf.* Ref. 3) by a decrease in viscosity of the solution because it is known that the viscosity of water decreases monotonously with temperature.[4b]

It should be emphasized that no analogous jumps were observed in the spectra of solutions of methyl glycosides of oligosaccharides. The "widths" of the transformed signals for the anomeric protons in the spectra of 0.2 *M* aqueous (D_2O) solutions of α-D-Glc*p*OMe, α-D-Man*p*OMe, α-D-Gal*p*OMe, β-D-Gal*p*OMe, and α-D-Gal*p*UA(OMe)OMe were independent of (to within the experimental error) the temperature in the range of 293—343 K. This suggests that a jumpwise change in one of the physical characteristics of a solution of allyl glycoside (**1**) reflects the changes in the state of the solution as a whole rather than is associated exclusively with anomalous behavior of water as the solvent.[4,5]

We believe that the above-described effect cannot be explained by the change of the conformational composition in solution described by the Boltzman distribution, because if this were the case, the properties would change gradually rather than jumpwise with temperature. Moreover, a change in the temperature left the overall spectral pattern of allyl lactoside (**1**) unchanged, and no signals, which could be assigned to different (distinguishable) conformers, were observed.

Apparently, the observed effect indicates that a process related to a phase transition occurs in solution. The fact that the "phase transition" actually takes place in an aqueous solution of compound **1** in the temperature range of 300—303 K is additionally confirmed by the presence of a jump (break) in the plot of the optical rotation ($[\alpha]_D$; measured on a JASCO DIP-360 digital polarimeter) for a 0.2 *M* aqueous solution of **1** *vs.* the temperature in the same temperature range (Fig. 1, *b*). When the concentration of **1** was doubled, this break became less pronounced, if at all (Fig. 1, *c*). Reliable results for more dilute solutions of **1** could not be obtained because of the small absolute value of specific rotation of allyl glycoside (**1**).

The "phase transition" found by us seems not to be related directly to the micelle formation well known for higher (C_8—C_{12}) alkyl glycosides. The extrapolation of the known critical micellization concentrations[6] of alkyl glucosides ($GlcOC_nH_{2n+2}$, n = 8, 10, or 12) to compounds with aglycons comprising the C_2—C_6 alkyl chains unambiguously indicates that already butyl glucoside does not possess any surfactant properties.

The newly found effect of a sharp change in two independent physical properties of an aqueous solution

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* The processing of the spectra included: 1) zero-filling (16K → 32K); 2) Lorentz—Gauss multiplication (LB = –2, GB = 0.1); 3) Fourier transformation.

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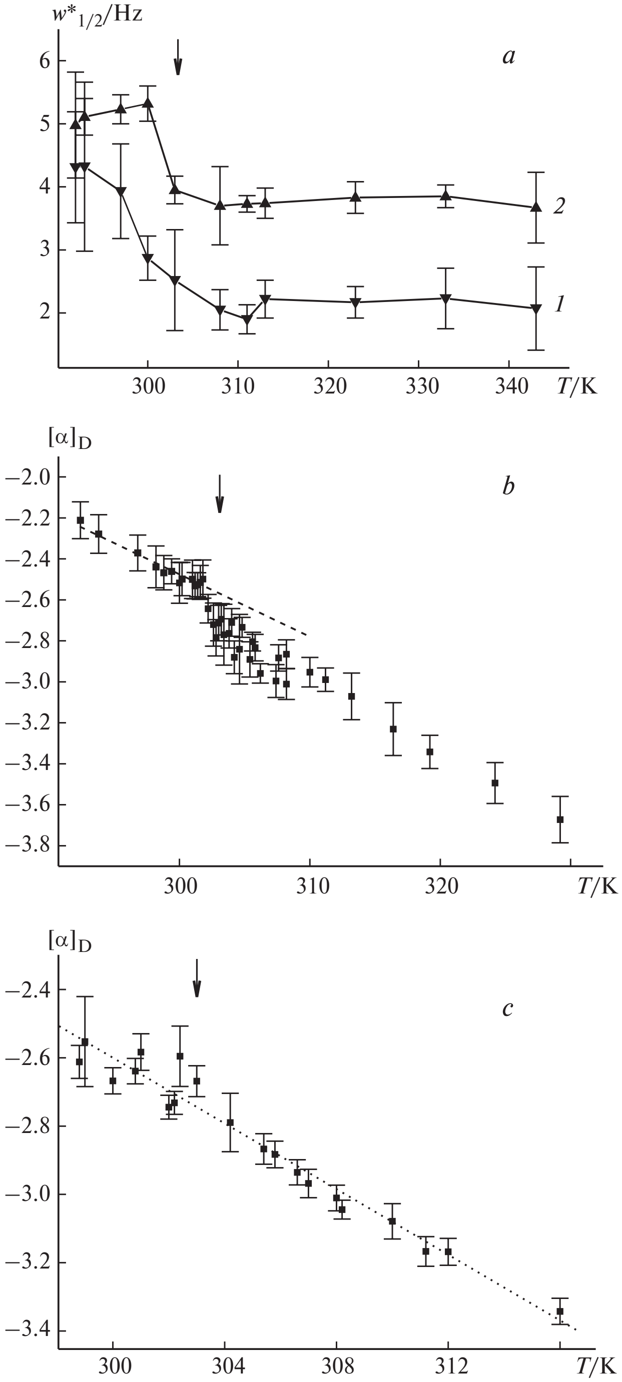

Fig. 1. *a*. Plot of the "width" ($w^*_{1/2}$) of the transformed (see the text) signals in the ^{1}H NMR spectrum of a 0.2 *M* solution of compound **1** in D_2O at δ 4.50 (*1*) and 5.25 (*2*) *vs*. the temperature (*T*); the temperature was maintained with an accuracy of ±1 K. *b*, *c*. The temperature dependence of the specific rotation ($[\alpha]_D$) of 0.2 *M* (*b*) and 0.4 *M* (*c*) solutions of **1** in H_2O; the temperature was maintained with an accuracy of ±0.2 K. The standard deviations were calculated using the *t*-Student distribution (95% probability).[9] The temperature of 303 K is indicated by an arrow.

of **1**, which we tentatively assigned to the "phase transition," might be related to the molecular aggregation of a solute (including sucrose[7]) in aqueous solutions to form clusters. The features and the very possibility of such a cluster formation have been discussed in recent years.[7,8] Judging from the published data, the solutions of compound **1** used in the present study could contain an enormous number of large-size clusters. A change in the temperature of the solution or its concentration may lead to a change in the composition or/and the structure of these clusters, which would necessarily be manifested in changes in some properties of the solution. We believe that we were lucky to observe such a rearrangement of clusters in a solution of allyl lactoside (**1**). It is also possible that the reasons for the effect under consideration are analogous to those responsible for carbohydrate—carbohydrate interactions, which are believed[1b] to be associated with very important biological functions. However, real physical interactions responsible for the apparent "phase transition" in an aqueous solution of allyl lactoside still remain unknown.

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