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Note

Complex formation between guar D-galacto-D-mannan and borate ion. Thermodynamic data

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The formation of complexes between borate ion and polyhydroxy compounds has been recognized for a long time[1]. The structural explanation of the complexation mostly accepted postulates that 1,2- and 1,3-diols of *cis* configuration form two types of complexes having the structure of diesters and being characterized by the great speed of their formation[2]. The 1:1 monocomplex between a molecule of polyol and a $B(OH)_4^-$ ion is formed by the expulsion of two molecules of water, and the 2:1 dicomplex by removal of four molecules of water from two molecules of polyol and a borate ion. The equilibrium constants for complex formation are defined by K_1 and K_2, respectively.

In two previous papers[3,4], we have presented ^{1}H-, ^{13}C-, and ^{11}B-n.m.r. data for borate complexes of a guar D-galacto-D-mannan polymer and related model compounds in alkaline solution. For each compound, the prevailing borate mono- and di-complexes were identified and, when possible, the associated formation constants determined[4].

We report herein some new ^{11}B-n.m.r. results concerning the thermodynamic data of the complexes on the basis of the temperature-dependence of the stability constants, both for model compounds and for the guar galactomannan polymer. As already mentioned, ^{11}B-n.m.r. spectroscopy provides a direct measurement of the amount of boron in the different environments[5], since discrete resonances can be observed for 1:1 and 2:1 anionic complexes with both 1,2- and 1,3-diols.

^{11}B-N.m.r. experiments were performed under the same conditions as previously described[4]. In particular, the pH was kept constant at 12 by use of a hydroxide–chloride buffer solution. Several model compounds were studied: methyl α-D-glucopyranoside, methyl β-D-mannopyranoside, and methyl α-D-galactopyranoside, as well as guar galactomannan fractions obtained after incubation with *A. niger* β-D-mannanase, followed by centrifugation and ethanol precipitation.

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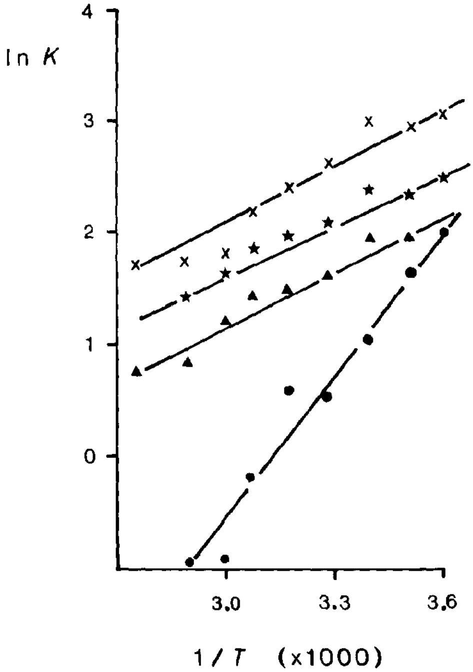


Fig. 1. Plot of ln$K$ *vs.* 1/T for borate–methyl α-D-galactopyranoside complexes: (★) 5-membered ring monocomplex; (▲) 6-membered ring monocomplex; (**X**) 5-membered ring dicomplex; and (●) 6-membered ring dicomplex. The straight lines represent the best fit obtained by linear regression calculation.

Complexation of glycoside model compounds with borate. — For each glycoside, a solution of known concentration of borate and of sugar was prepared and examined by ^{11}B-n.m.r. spectroscopy over a temperature range from 278 up to 355 K. At low temperature, all corresponding ^{11}B-spectra showed sharp and well-resolved resonances, which were assigned according to previous works[5]. Upon temperature variation, chemical shift and linewidth changes could be observed. Thus, a slight low-field linear shift was apparent for all signals, except for the line corresponding to the free borate ion, which moved more strongly in the same direction. This general downfield shift could be accounted for by the rising acidity of the medium upon temperature increase. Linewidths were also affected by the temperature increase, and the same trend was observed for all model compounds. The free borate ion and the 6-membered ring monocomplex lines became wider. On the contrary, the 5- and 6-membered ring dicomplexes displayed sharper signals. At the same time, the 5-membered ring monocomplex resonance showed a more complex behavior with a linewidth that decreased up to 320–325 K, and then increased at higher temperatures. Changes in the viscosity and pH of the medium, as well as modification of the occurrence of diastereoisomeric borate diesters which resonate at almost the same frequency, would explain these phenomena. The formation constants of the various complexes involved were determined, as previously

TABLE I

ASSOCIATION CONSTANTS AND THERMODYNAMIC DATA OF MODEL COMPOUNDS

Compound	*Nature of the complex*	K_1 *or* K_2 *(295 K)*	$\Delta H°$ *(kJ·mol^{-1})*	$\Delta S°$ *(J·mol^{-1}·K^{-1})*
Methyl α-D-glucopyranoside	4,6-mono-	2.1	−8.0	−21.1
	4,6-di-	0.14	−16.3	−71.4
Methyl β-D-mannopyranoside	2,3-mono-	5.3	−6.1	−6.9
	2,3-di-	3.6	−7.2	−13.7
	4,6-mono-	3.5	−15.8	−43.1
Methyl α-D-galactopyranoside	3,4-mono-	9.5	−12.5	−23.8
	3,4-di-	16.1	−15.9	−30.8
	4,6-mono-	6.3	−14.3	−32.9
	4,6-di-	3.0	−35.6	−111.0

described[4], from peak areas. This required the help of a bandshape-simulation program, in the case of overlapping signals. Some scattering in the calculated value could sometimes be introduced because of the difficulty to estimate properly the occurrence of very minor species, such as the 6-membered ring dicomplex and the mixed spirocyclic dicomplex.

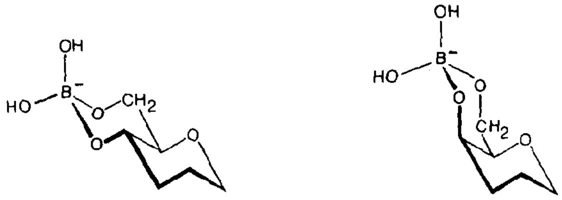
Plots of $\ln(K_1$ or $K_2)$ *vs.* $1/T$ for methyl α-D-galactopyranoside is presented, as an example, in Fig. 1. A strong tendency towards a linear dependency clearly appears for each (K_1 or K_2) value. According to the general relationship (*1*), the thermodynamic data, $\Delta H°$ and $\Delta S°$, could be considered as constants over the studied temperature range and thus determined (Table I).

$$-\frac{\Delta G°}{RT} = \ln K = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R} \qquad (1)$$

From the values obtained, some general features could be drawn. As previously reported for borate–glycol[6], borate–polyols[7], and borate–mannitol[8] complexes, all $\Delta H°$ and $\Delta S°$ values are negative. The formation of all stable complexes is exothermal. The negative-entropy change, $\Delta S°$, indicated that any entropy gain from the liberation of water from both the borate and the glycoside does not exceed the entropy loss due to the complex formation[9].

The 6-membered ring complexes involving O-4 and O-6 could be divided into two groups according to the equatorial or axial orientation of O-4. On borate complexation, these led to *trans*- or *cis*-fused rings, respectively (see Fig. 2).

The corresponding values of the formation constants, both for mono- and di-complexes, showed that the *cis*-position leads to more stable complexes. The case of 6-membered ring dicomplexes, even if they are of minor occurrence when in competition with 5-membered ring dicomplexes, is particularly representative of this tendency.



trans-fused *cis*-fused

Fig. 2. The *trans*- and *cis*-fused rings obtained in the case of 6-membered ring complexes.

Moreover, as mentioned by Foster[10], for the *trans*-fused group the 6-membered ring monocomplex of the α anomer is destabilized as compared to the β anomer. This is reflected by the respective ΔH° values corresponding to methyl α-D-glucopyranoside and methyl β-D-mannopyranoside. For methyl α-D-glucopyranoside, the glycosidic methoxyl group occupies an axial position and will interact strongly with the axial hydrogen atoms at C-3 and C-5. Still, in the case of the *trans*-fused family, 6-membered ring dicomplexes are hardly detectable for both mannopyranoside anomers[4]. Also, thermodynamic data for a methyl α-D-glucopyranoside dicomplex showed an important entropy loss leading to a weak value of the association constant.

The case of methyl α-D-galactopyranoside is quite different, with a strong negative enthalpy variation balanced with a very important entropy loss. This leads to a 6-membered-ring dicomplex stable at low temperature but very sensitive to any temperature increase. A similar behavior has been found for 6-membered ring dicomplexes between poly(vinyl alcohol) and borate ion[9].

The presence of 5-membered ring complexes depends on the occurrence of two adjacent hydroxyl groups in a *cis* configuration. This is the case for OH-2 and OH-3 of the D-mannose units, as well as OH-3 and OH-4 of the D-galactose units. The comparison between the association constants shown in Table I points out that the 5-membered ring complexes obtained for **3** are more stable than those for **2**. This could be due to the interference of the equatorial glycosidic methoxyl group for **2**, which hinders the complex formation[10]. On the contrary, the values previously obtained[4] for methyl α-D-mannopyranoside fit much better (K_1 ~15 for 2,3-monocomplex and K_2 ~50 for 2,3-dicomplex) and support this explanation, as the glycosidic methoxyl group, in axial position, does not interfere with the complex formation.

Complexation of galactomannan fractions with borate. — ^{11}B-N.m.r. spectroscopy. Compared to the spectra of model compounds, the spectra obtained with fractions of low-molecular-weight guar galactomannan showed in general wider and less well-defined lines. These features are certainly the result of a higher viscosity of the guar solutions, and of the existence of several isomeric complexes in which the boron environments are almost but not exactly identical. Nevertheless, the occurrence of mono- and di-complexes having 5- and 6-membered rings has already

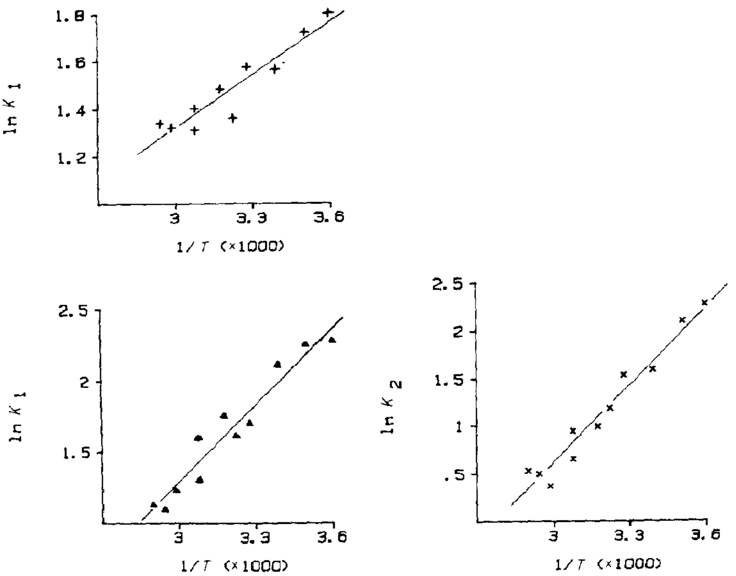


Fig. 3. Plots of ln K *vs.* $1/T$ for borate–galactomannan fraction complexes: (+) 5-membered ring monocomplex; (▲) 6-membered ring monocomplex; and (×) 5-membered ring dicomplex. Best fit obtained by linear regression calculation.

TABLE II

ASSOCIATION CONSTANTS AND THERMODYNAMIC DATA OF GUAR GALACTOMANNAN[a]

Nature of the complex	K_1 *or* K_2 *(295 K)*	$\Delta H°$ *(kJ·mol⁻¹)*	$\Delta S°$ *(J·mol⁻¹·K⁻¹)*
4,6-Mono-	7.4	−15.1	−34.6
5-Membered ring mono-	4.9	−6.1	−7.3
5-Membered ring di-	5.4	−22.9	−63.7

[a] $[B^-] = 3.63$mM; $[S] = 0.3$M.

been noticed[4]. However, the distinction between complexes involving galactopyranosyl or mannopyranosyl units has never been achieved. The temperature-dependence of the chemical shifts followed the same low-field shift as that of the model compounds. With increasing temperature, the linewidth of the free borate ion and the 5-membered-ring monocomplex showed little variation. On the contrary, a flattening occurred for the lines of the 6-membered-ring monocomplex and the 5-membered-ring dicomplex.

Association constants and thermodynamic data. — The association constants of the three main complexes formed by guar fractions were determined as previously described[4]. Plots of ln K *vs.* T^{-1} are presented in Fig. 3. In spite of an important scattering of the experimental points, the estimation of the thermodynamic parameters was still possible over the temperature range studied (see Table II). As the 6-membered-ring complex involves only galactosyl residues, the corresponding association constant K_1 was calculated while taking into account the galactosyl content of the guar sample. The results are indeed very close to the values obtained for methyl α-D-galactopyranoside, pointing out the parallel behavior towards monocomplexation of the galactose units, either in a polysaccharide chain or in a monosaccharide derivative.

On the contrary, 5-membered-ring mono- and di-complexes can involve both galactosyl and mannosyl units. In the calculation of the association constants, it could be shown that the replacement of the total concentration of free glycosyl units by the concentration of either galactosyl or mannosyl units led to the same ΔH° value (provided the occurrence of complexes molecules is low as compared to the occurrence of free molecules). This is represented only by a vertical shift of the plot of $\ln K$ *vs.* T^{-1}.

In the case of the 5-membered-ring monocomplex, all the values determined at 295 K were in close agreement with the values obtained for methyl β-D-mannopyranoside. This suggested that this type of monocomplexation predominates for the backbone mannosyl units rather than for the galactosyl side-units.

The ΔH° value obtained for the 5-membered-ring dicomplex is closer to the one determined for methyl α-D-galactopyranoside than for methyl β-D-mannopyranoside. This, with the existence of a specific polymer effect towards dicomplexation (as compared to dicomplexation involving model compounds), could imply a selective involvement of galactosyl side units during dicomplexation. This finding fully agrees with recent results of Winter *et al.*[11] concerning the molecular organization of galactomannans from different plant sources. By diffraction methods and molecular modeling, these authors have deduced a generalized model where all galactomannans from mannan to fenugreek gum (with a high degree of galactose substitution) exhibit the same sheet-like structure, stabilized by mannan–mannan intermolecular hydrogen bonds and having the galactosyl units defining the accessible sheet surface. On addition of borate ion, mono- and di-complexation occur. The latter, which corresponds to cross-linking of the polysaccharide chains, leads to the gelation phenomenon *via* the molecular basis just described.

EXPERIMENTAL

Materials. — Methyl α-D-glucopyranoside and methyl α-D-galactopyranoside were commercial compounds from Sigma Chemical Co. Methyl β-D-mannopyranoside was prepared in this institute according to a glycosylation reaction catalyzed by hydrogen fluoride[12]. Guar *(Cyamopsis tetragonolobus)* D-galacto-D-mannan was prepared and purified from commercial flour. It contained D-galactose and D-mannose in the molar ratio of 19:31. To produce low-molecular-weight oligomers, samples (4 g/L) were redissolved in 10mM acetate buffer (pH 4.5) and treated with highly purified *A. niger* β-D-mannanase[13] (50 μL). The solution was first incubated for 4 h at 40°, and then, in order to inactivate the enzyme, another incubation was performed for 10 min at 100°. The unreacted solid material was removed by centrifugation at 15 000 r.p.m. for 30 min. An appropriate amount of ethanol was added to obtain 40 and 75% solutions, and the guar fractions were recovered after centrifugation at 15 000 r.p.m. for 30 min. Borate solutions were prepared with D_2O and $NaBO_2 \cdot 4\,H_2O$ from BDH Chemicals.

^{11}B-N.m.r. spectroscopy. — Weighed quantities of glycosides [400 mg for methyl α-D-glucopyranoside (**1**), 150 mg for methyl β-D-mannopyranoside (**2**) and methyl α-D-galactopyranoside (**3**)] or depolymerized guar fractions (~130 mg) were dissolved in a hydroxide–chloride buffer solution (pH 12) in the presence of borate solution of known concentration (36.3mM for **1**; 72.6mM for **2** and **3**; 3.63mM for guar fractions). The volume was adjusted to 2.5 mL. The ^{11}B-n.m.r. spectra were recorded at 96.28 MHz with a Bruker spectrometer, equipped with an Aspect 3000 computer. The temperature was varied from 278 to 355 K. Ethyl ether–BF_3 was used as external reference. The spectra were obtained in the F.t. mode with a spectral width of 4000 Hz, 8 k data points, and a pulse width of 15 μs (90° pulse) giving a resolution of 0.961 Hz per point. The signals, which were in general well resolved, were integrated by use of the facilities of the spectrometer or with the help of a band-shape simulation program.

Calculation of equilibrium constants. — The values of $[B^-]$, $[BS^-]$, and $[BS_2^-]$ could all be determined directly from the peak areas; $[S]$ was the concentration of uncomplexed sugar and was calculated according to Eq. (*2*), where $[S]_o$ is the initial concentration of sugar.

$$[S]_o = [S] + [BS^-] + 2[BS_2^-] \qquad (2)$$

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