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NUCLEAR MAGNETIC RESONANCE EVIDENCE OF THE GUAR–BORATE INTERACTION

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ABSTRACT

The formation of borate complexes, on addition of sodium tetraborate to solutions of guar galactomannan in D_2O, was studied by ^{13}C-n.m.r. spectroscopy. From the changes observed, some conclusions concerning the molecular motion of this polysaccharide and the preferential sites of complexation have been drawn. These conclusions, which were substantiated by an ^1H-n.m.r. study of borate–methyl α-D-galactopyranoside complexes in di($^2H_3$)methyl sulfoxide, may serve as a basis for understanding guar galactomannan–borate gelation.

INTRODUCTION

The ability of the borate ion to react with polyhydroxy and related compounds has been known and used for a long time[1,2]. Borate complexes can exist in many forms as cyclic esters and ionized, strongly acidic complexes in which one boron atom is associated with one or two molecules of a diol[3,4] (or as a tridentate type formed with cyclitols[5] and with derivatives in the mannose series[4]). The special requirements for their formation has provided a highly useful means for examining stereochemical and structural problems[6,7].

Several methods have been used to investigate borate-complexing reactions, such as conductivity, pH measurements, cryoscopic studies, and also polarimetry[8] and zone electrophoresis[7]. Mazurek and Perlin[3] have studied borate complexing in five-membered-ring, vicinal diols from vapor-pressure equilibrium and n.m.r. spectral observations. Also, the detection of complex formation by ^{13}C-magnetic resonance spectroscopy was described by Gorin and Mazurek[4].

The present study deals with the case of a more complex system, *i.e.*, the legume-seed D-galacto-D-mannan from guar. This polysaccharide consists of linear chains of (1→4)-linked β-D-mannopyranosyl residues, to which are attached (1→6)-linked α-D-galactopyranosyl group (as single-unit side-chains) at O-6 of certain D-mannopyranosyl units. Because of its viscosity properties, guar is extensively exploited in various commercial applications[9]. On addition of sodium tetraborate at alkaline pH, gels are formed from guar or guar derivatives, and more generally

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from a number of polysaccharides containing hydroxyl groups in a favorable position to react with the borate ion (the hydroxyl groups should be adjacent and *cis*). This is the case for hydroxyl groups occurring at C-2 and C-3 of the D-mannose units, as well as at C-3 and C-4 of the D-galactose units.

Gelation is supposed to take place by cross-linking of different polymer chains, or sometimes parts of the same chain with borate, in such a way that a three-dimensional network of connected chains is formed. When the concentration of cross-linked chains is high, solvent is to a large extent immobilized in the network and a semisolid gel results. Although it has been hypothesized, this model has never been firmly established.

In this study, the borate–guar gum interaction has been investigated by ^{13}C-nuclear magnetic resonance spectroscopy. The findings could provide some new insights concerning the molecular interactions involved during complex formation. Also and more generally, they could serve as a basis for the understanding of guar–borate gelation.

EXPERIMENTAL

Preparation of galactomannans. — Guar *(Cyamopsis tetragonolobus)* and carob *(Ceratonia siliqua)* D-galacto-D-mannans were prepared from the commercial flours according to the method of McCleary *et al.*[10]. They contain D-galactose and D-mannose in the molar proportions 19:31 and 19:81, respectively. Samples were redissolved in cold water after being ground with a mortar and pestle at liquid N_2 temperatures. After centrifugation at 15 000 r.p.m. and 4°, the solution was lyophilized. The product obtained was stored at −18°.

N.m.r. spectroscopy. — Samples of guar galactomannan and methyl α-D-galactopyranoside were dissolved in D_2O (30 mg/mL) and di($^2H_3$)methyl sulfoxide (60 mg/mL), respectively. The deuterium resonance was used as the field-frequency lock. Experiments were performed at 75 MHz for ^{13}C-n.m.r. spectra and at 400 MHz for ^1H-n.m.r. spectra with Bruker spectrometers equipped with an Aspect 3000 computer. ^{13}C-spectra were obtained in the F.t. mode at 333°K, by use of a spectral width of 4000 Hz and a resolution of 0.484 Hz per point. ^{13}C-n.m.r. assignments were made with reference to the reports of Grasdalen and Painter[12], and McCleary *et al.*[10]. All ^{13}C-n.m.r. T_1 measurements were performed by use of the inversion-recovery method with delay times of a least 8 T_1 values and ^1H decoupling. T_1 values were determined by use of the automatic T_1 calculation program provided by Bruker (±5%). Nuclear Overhauser enhancements (n.O.e.) were derived from the ratio of the intensity of fully decoupled spectra to the intensity of spectra in which the proton-noise decoupler was gated off during at least 8 T_1 values, to eliminate the n.O.e. effect. The estimated error was 5%. The ^{13}C-n.m.r. T_2 measurements were performed by the Hahn-spin-echo sequence again with complete noise decoupling of protons. T_2 values were calculated by use of the T_2 calculation routine from Bruker. Under these experimental conditions, the values ob-

tained were lower than the true values owing to incoherent decoupling[13]. This led to a very rapid decay of the transverse magnetization. Possible remedies would require coherent proton irradiation[14]. Another approach to this problem is to calculate a corrected T_2 value as performed by Benesi and Brant[15]. Neither of these solutions was used here. ^{1}H-N.m.r. spectra were acquired at 400 MHz with a spectral width of 1200 Hz and 32 k data points (resolution, 0.073 Hz per point). Chemical shifts are expressed in δ values downfield from the signal of di($^2H_3$)methyl sulfoxide (δ 2.49, Me_4Si). Hydroxyl protons were assigned according to Gillet *et al.*[11].

Borate addition. — All borate additions were accomplished with commercial $Na_2B_4O_7 \cdot 10\ H_2O$, which was repeatedly evaporated with D_2O prior to use. For ^{13}C-n.m.r. spectroscopy, solutions containing D_2O (2 mL), guar sample (60 mg), and successive additions of 8 mg (0.02M), 16 mg (0.04M), and 32 mg (0.08M) were prepared. T_1 and T_2 measurements were performed with 15 mg of deuterated borate. For ^{1}H-n.m.r. spectroscopy, solutions containing di($^2H_3$)methyl sulfoxide (0.5 mL), methyl α-D-galactopyranoside (30 mg), and successive quantities of deuterated borate (3.5, 5.0 mg) were prepared at least one night in advance prior to use.

Viscosity measurements. — Intrinsic viscosity was measured with an Ubbelohde viscosimeter. The relative-viscosity dependence of the guar samples on borate concentration was established, at 25° with a Low–Shear 30 (Contraves) viscosimeter, by monitoring the relative viscosity of a solution of guar fraction (30 mg/mL) after addition of sodium tetraborate (50 mg/mL) at zero-shear rate. At a borate concentration higher than 3 mg/mL, the viscosity of the polymer solution is such that no measurement was possible.

RESULTS AND DISCUSSION

^{13}C-N.m.r. spectroscopy of guar complexation with borate. — According to Grasdalen and Painter[12], the key to successful n.m.r. spectroscopy of galactomannan samples has proved to be an initial, limited depolymerization which diminishes the viscosity of their solutions and improves their solubility. Partial acid hydrolysis[12,16] that is mild enough not to affect the structure of the polysaccharide is one answer. Another is the selective enzymic cleavage or particular preparation of the sample, including redissolution of the flour in cold water after grinding with a mortar and pestle at liquid nitrogen temperature. The latter procedure was used in this study and led to samples that could be easily utilized for n.m.r. studies with no change in the mannose-to-galactose ratio. An intrinsic viscosity of 65 $mL \cdot g^{-1}$ was measured and a weight-average mol. wt. of 100 000 was estimated from the Mark–Houwink relationship.

Fig. 1 shows the ^{13}C-n.m.r. spectra obtained at 60° after progressive addition of sodium tetraborate to a D_2O solution of guar galactomannan (galactose-to-mannose ratio of 19:31). The most striking effect with increasing concentrations of

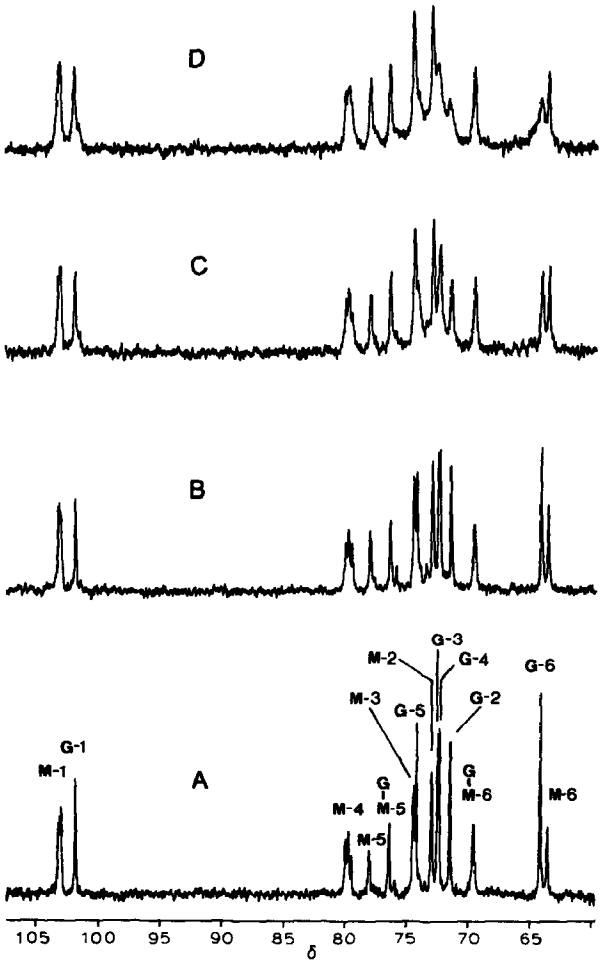

Fig. 1. ^{13}C-n.m.r. spectra (75 MHz) of solutions of guar galactomannan (30 mg/mL) in D_2O at 60°: (A) Without sodium tetraborate; (B) with deuterated sodium tetraborate (8 mg); (C) with deuterated sodium tetraborate (16 mg); and (D) with deuterated sodium tetraborate (32 mg). Abbreviations: M, Man; G, Gal.

borate was a drastic change in the displayed intensity of all the carbon atoms of the (1→6)-linked α-D-galactopyranosyl groups. This phenomenon seemed to be selective, as the carbon atom signals of the (1→4)-linked β-D-mannopyranosyl residues appeared to be broadened only slightly, which corresponded to changes in the viscosity of the medium. The same phenomenon was seen when carob D-galacto-D-mannan (galactose-to-mannose ratio of 19:81) was treated with increasing concentrations of sodium tetraborate. On the contrary, sodium carbonate or sodium hydroxide appeared to have no effect on the resonances and intensities of the signals observed in the case of guar galactomannan.

Gorin *et al.*[4] have already shown that, after addition of sodium tetraborate to

TABLE I

^{13}C-SPIN-LATTICE RELAXATION TIMES AND NUCLEAR OVERHAUSER ENHANCEMENT (n.O.e.) OF GUAR D-GALACTO-D-MANNAN[a]

Carbon atom[b]	*n.O.e.*	T_1[c]	
		Without sodium tetraborate	*With sodium tetraborate (15 mg)*
Man-1	1.85	0.33	0.32
	1.94	0.28	0.28
Gal-1	2.10	0.46	0.40
Man-4[d]	1.82	0.26	0.26
	1.79	0.31	0.25
	1.80	0.38	0.32
Man-5	1.97	0.31	0.31
Gal→Man-5	1.94	0.26	0.25
Man-3	1.94	0.30	0.30
Gal-5	2.23	0.40	0.34
Man-2	1.96	0.28	0.28
Gal-3	2.22	0.43	0.40
Gal-4	2.13	0.47	0.41
Gal-2	2.29	0.42	0.40
Gal→Man-6	1.69	0.13	0.13
Gal-6	2.51	0.48	0.40
Man-6	2.03	0.17	0.17

[a]Measured at 75 MHz and at 333°K for a 30 mg/mL solution in D_2O. [b]Carbon-atom assignments according to their appearance from low to high field. [c]T_1 values are expressed in seconds. [d]This splitting of lines is explained by assuming that the C-4 (Man) resonance is influenced by the presence of a branch on the preceding residue linked to O-4[12].

solutions of carbohydrates in D_2O, the formation of borate complexes could be monitored by changes in the ^{13}C-n.m.r. spectra. Two types of spectral effects were observed. In one type, the signals remained sharp and changes in chemical shift took place and, in the other, the resonances of the signals remained the same, but a broadening of the signals of ^{13}C nuclei in the vicinity of the complex occurred. Two explanations for the latter phenomenon which involved conformational factors were considered[4]. Most of the broadening was due rather to either the presence of more than one conformer of the borate complex or to the rapid interconversion of the compound with borate complexes of different types[4].

Relaxation measurements. — In the present study, the phenomenon was observed by relaxation measurements (spin–lattice and spin–spin relaxation times, T_1 and T_2) for the polysaccharide carbon atoms, in the absence and presence of borate, in order to see whether further information concerning the interaction could be drawn. The study was performed at 60° to decrease the viscosity and, thereby, the line widths. Thus, relaxation measurements were made possible. However, as shown by spectra obtained at 30°, the phenomenon remained essentially the same (see Tables I and II). One evident conclusion is that the largest effect observed in

TABLE II

^{13}C-SPIN-SPIN RELAXATION TIMES OF GUAR GALACTOMANNAN[a]

Carbon atom[b]	T_2[c]	
	Without sodium tetraborate	*With sodium tetraborate (15 mg)*
Man-1	0.032	
	0.027	0.019
Gal-1	0.035	0.014
Man[d]-4	0.022	0.014
	0.023	0.014
	0.032	0.015
Man-5	0.035	0.022
Gal→Man-5	0.028	0.017
Man-3	0.026	0.021
Gal-5	0.038	0.016
Man-2	0.026	0.021
Gal-3	0.039	0.015
Gal-4	0.038	0.015
Gal-2	0.045	0.015
Gal→Man-6	0.022	0.014
Gal-6	0.045	0.015
Man-6	0.023	0.015

[a-d]See footnote to Table I.

terms of spin–spin relaxation-time variations is experienced by the carbon atoms of the galactose residues (Table II). However, some sequence-related heterogeneity of chemical shifts could also be taken into account to explain a noticeable line width variation observed for C-4 (Man), C-5 (Man), and C-5 (branched Man residue). Of course, on addition of more sodium tetraborate the T_2 values would vary much more as could be estimated indirectly from the linewidth $\Delta\nu_{0.5}$ (see Fig. 1) by means of relationship (*1*),

$$T_2^* = 1/\pi \cdot \Delta\nu_{0.5} \qquad (1)$$

where T_2^* refers to effective relaxation-time, including a field inhomogeneity contribution. By contrast, T_1 values (Table I) did not show such strong variations (at least at this borate concentration) for carbon atoms of the galactose residues. Nevertheless, the phenomenon exists and seems to be more selective, as C-1, C-4, C-5, and C-6 (all from the galactose residues) are the most influenced.

In the absence of borate ion, the n.m.r. data were consistent with the typical behavior of random-coil polysaccharides in solution with T_2 values ~25–30 ms for carbon atoms of the mannose residues, and ~40 ms for those of the galactose units (see Experimental section for comments). T_1 values were also always longer for the latter and this may reflect a higher reorientational mobility of the single-unit side-chains, compared to lower flexibility of the main chain.

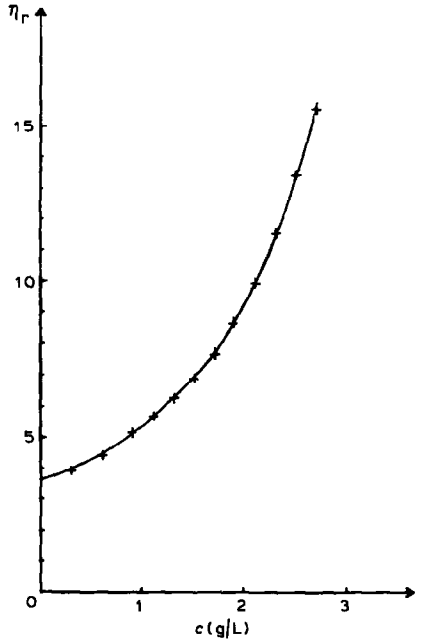

Fig. 2. Relative viscosity-dependence of guar galactomannan (30 mg/mL) on borate concentration at zero-shear rate.

In the presence of borate ion, this local mobility could be completely lost. Following interaction between galactose units and borate ions, stiffening of the side-chain segments occurred. This stiffness may become so important that the (1→6)-linkages are entirely blocked and the corresponding line widths so great that all galactose peaks are broadened into the baseline of the spectrum. In comparison, the flexibility of the main chain does not change appreciably (as shown by T_1 values of carbon atoms of the mannosyl residues). Also, the strong increase in viscosity of the solution (see Fig. 2) should be taken into account to explain the general shortening of T_2 values relative to the carbon atoms of the main chain.

Interaction of sugar models with borate. — In order to gain an understanding of the phenomenon described above, sugar models (methyl β-D-mannopyranoside and methyl α-D-galactopyranoside) were investigated. In the presence of the easily accessible methyl α-D-galactopyranoside, the ^{13}C-n.m.r. spectra showed both phenomena (slight changes in ^{13}C-chemical shifts and predominant broadening of signals) for solutions in water after addition of sodium tetraborate, for all hydroxyl-bearing carbon atoms (C-1 bearing a semi-acetal group was not affected). No clear conclusion concerning the interaction could be drawn from this result. Consequently, ^{1}H-n.m.r. spectroscopy of the sugar model in di($^2H_3$)methyl sulfoxide was studied. This aprotic solvent was chosen because the rate of exchange in it is usually slow, which allows not only a separate observation of the slow-exchangeable hydroxyl protons, but also a possible identification of borate complexes of different

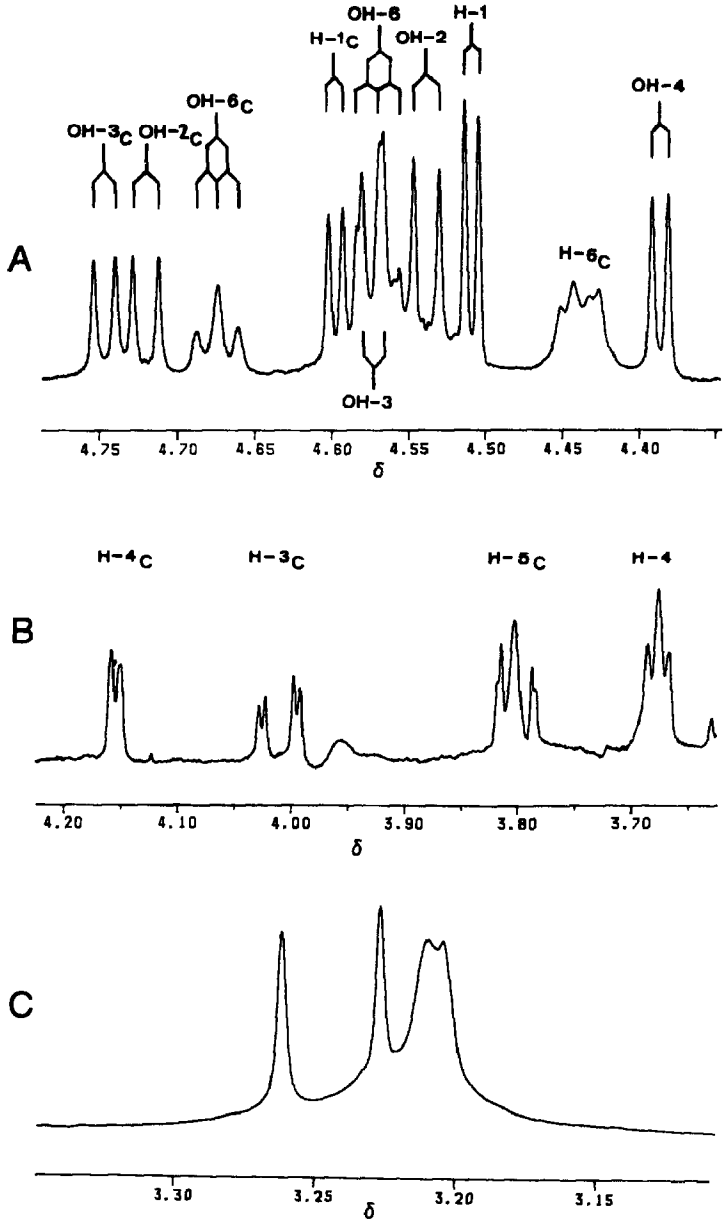

Fig. 3. Partial 400-MHz, ^{1}H-n.m.r. spectra of methyl α-D-galactopyranoside (60 mg/mL) after addition of deuterated sodium tetraborate (10 mg/mL) in di ($^2H_3$)methyl sulfoxide.

types. However, owing to the low solubility of sodium tetraborate into di($^2H_3$)methyl sulfoxide, only small quantities of borate and methyl α-D-galactopyranoside could be mixed. Fig. 3 shows different parts of the spectrum obtained at 295°K. In the resonance region of methoxyl protons, three or four signals (de-

TABLE III

CHEMICAL SHIFTS AND VICINAL H–O–C–H COUPLING CONSTANTS OF THE HYDROXYL PROTONS OF METHYL α-D-GALACTOPYRANOSIDE[a] IN DI($^2H_3$)METHYL SULFOXIDE

	OH-2	*OH-3*	*OH-4*	*OH-6*
Chemical shift (δ)	4.491	4.518	4.340	4.541
Δδ (Hz) after addition of sodium tetraborate[b]	18.8	21.2	18.0	10.4
$J_{H-C-O-H}$ (Hz)	6.6	5.7	4.3	5.1, 6.0[c]

[a]As a solution (60 mg/mL) at 295°K. [b]Low-field shift of hydroxyl protons of the still uncomplexed sugar after addition of sodium tetraborate (10 mg/mL). [c]In some well-resolved spectra, two couplings were detected for OH-6.

pending of the borate concentration) are seen. Starting from the signal at δ 3.23, which corresponds to the chemical shift of the methyl protons in the uncomplexed sugar, one additional signal appeared at δ 3.27 and another one at high field (δ 3.21). After increasing the borate concentration, one additional signal was seen at δ 3.22. Thus, it appears that, at this borate concentration, at least four compounds are present (Fig. 3C).

Fig. 3A shows the hydroxyl proton region. Even when the hydroxyl protons of the uncomplexed sugar were shifted to low-field in the spectrum of the borated derivative, they were still easily recognizable by reference to their characteristic vicinal-coupling constants. These vicinal H–C–O–H coupling constants and the chemical shifts are listed in Table III. In this region are also the resonances of H-1 at δ 4.507 (uncomplexed molecule) and at δ 4.596 (complexed molecule), with a low-field shift of 35.6 Hz between the signals of both molecules.

More interesting are the three signals that appeared between δ 4.6 and 4.8. These two doublets and one triplet were assigned, by means of the measured coupling-constants, to hydroxyl protons corresponding to OH-3, OH-2, and OH-6, respectively, originating from the complexed molecules. The most satisfactory assignment for these signals are to the 3,4- and 4,6-complexes, the existence of which has already been suggested by ^{11}B-n.m.r. data of solutions of sugars in the presence of borate ion[17]. The first complex has a five-membered ring that leaves free OH-2 and OH-6; the second has a six-membered ring complex with OH-2 and OH-3 free. In the first case, only the signal of OH-6 was clearly seen; OH-2 gave a resonance that is overlapped by other signals and was barely visible, even after variation of the temperature. Also, with a slight increase in temperature (to 302°K), it was possible to detect a small doublet at δ 4.379, whose chemical shift was temperature dependent and whose vicinal coupling-constant was 5.7 Hz. This resonance could correspond to the OH-3 proton of a dicomplex (one borate and two molecules of galactoside). If this assumption is true, the observed dicomplex would be a spirocyclic complex involving preponderantly O-4 and O-6.

Fig. 3B represents another interesting part of the spectrum obtained at 295°K. It shows the protons of the sugar ring, shifted to low field by the direct

influence of borate substitution as they are linked to the same carbon atom. These protons have chemical shifts that are temperature independent, their intensity being directly related to the concentration of borate ion. Another feature of this spectrum is that its resonances did not exhibit any vicinal H–C–O–H coupling-constant, which constitutes a good proof for the covalent character of the borate complex[18]. This is attributed to the loss of the hydroxyl proton during complexation, at least in this solvent. Thus, the resonances belonging to the monocomplexed molecules were those strongly shifted to low field, such as H-3 at δ 4.01, H-4 at δ 4.16, and H-6 at δ 4.4; H-5, which was less affected, resonated at δ 3.80. A typical shift of 0.48 p.p.m., introduced by the complexation phenomenon involving O-4, could be observed for H-4 on a sample in dimethyl sulfoxide.

Extension to the galactomannan polymer. — In a study of the borate–carbohydrate complex by means of refrative index and optical rotation measurements, Malcolm *et al.*[19] have determined the stability constants for those glycosides that are directly related to the D-galacto-D-mannan polymer. From these measurements, it appeared that the borate complex was formed more readily on the galactose side-units than on the mannose backbone. However, the cross-linking site was found to be likely on either the mannose or galactose units.

The present ^{13}C-n.m.r. data strongly agree with the first assumption, in particular when the noticeable influence in terms of line broadening of the galactose carbon signals after addition of borate is considered. Although both types of complexes (3,4 and 4,6) are possible, the T_1 values are in favor of a preponderant six-membered ring complex. This conclusion is supported on steric grounds since the galactose side-chain is relatively more accessible, as compared to the mannose backbone, and 4,6-complexes are more easily formed from glycosides[12] at pH ~10, which is approximately the pH at which the present experiment was performed.

For solutions in di($^2H_3$)methyl sulfoxide, the integral curves corresponding to the hydroxyl protons OH-3, OH-2, and OH-6, showed that the 3,4-complex had a slightly greater concentration than the 4,6-complex (27:23). This conclusion should hold true in the case of aqueous solutions, in particular if one considers that, after equilibrium, the main driving force for complex formation is the geometrical requirement of the hydroxyl groups as opposed to the influence of the solvent. The occurrence of both complexes is consistent with the overall change in the galactose carbon signals as described earlier. Based on steric considerations, the selective involvement of galactose side-units for borate-complex formation would be maintained during cross-linking of the chains leading to gelation.

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