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THE INTERACTION OF SUGARS WITH BORATE: AN N.M.R. SPECTROSCOPIC STUDY

G. R. KENNEDY* AND M. J. HOW**

Department of Chemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT (Great Britain)

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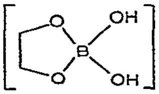
ABSTRACT

^{11}B n.m.r. spectroscopy studies of solutions of sugars in the presence of borax have shown the existence of boron-containing complexes. Chemical-shift values indicate the size of the ring in which boron is involved. Use of this method and p.m.r. spectroscopy showed that D-glucose forms a 1,2-furanoid and 1,2-pyranoid complex in the presence of borax and benzeneboronic acid.

INTRODUCTION

Many principles concerning the interaction of borate with hydroxy compounds were established by Böeseken[1], and borate complex formation has been used for analytical purposes in electrophoresis[2]. More recently, ^{11}B n.m.r. spectroscopy has confirmed that complex formation occurs between borax and a range of simple hydroxy compounds, *e.g.* ethane-1,2-diol, propane-1,3-diol, and pinacol, in solution and is dependent on pH, concentration, and structure[3]. The spectroscopic method of study does not disturb chemical equilibria and characteristic chemical-shift data indicate the existence of five- and six-membered ring complexes (**1** and **2**), and monocyclic and spirocyclic complexes (**3** and **4**). We now report the application of ^{11}B n.m.r. spectroscopy to a study of the interaction of carbohydrates with borate.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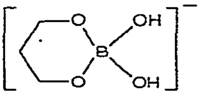
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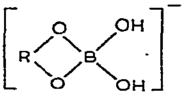
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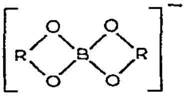
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RESULTS AND DISCUSSION

The ^{11}B n.m.r. spectra of diols complexed with borate show sharp, well-resolved signals[3], but the spectra for complexed carbohydrates are not as well-defined.

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*Present address: Fisons Ltd., Pharmaceutical Division, Research and Development Laboratories, Bakewell Road, Loughborough, Leics. LE11 OQY.

**Present address: Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, Beds.

TABLE I

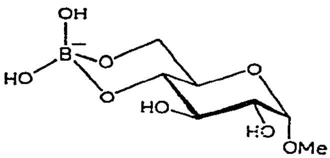
THE ^{11}B N.M.R. SPECTROSCOPIC DATA FOR SUGAR-BORAX SOLUTIONS AND THE DEDUCED RING-SIZE OF THE BORATE COMPLEX

Sugar	*Sugar–borate ratio*^{a}	*Borax concn.* (M)	*pH*	δ^b	W^c	A^d	δ^b	W^c	A^d	δ^b	W^c	A^d	*Ring size of complex*
Methyl α-D-galactopyranoside	0.75:1	0.25	12	2.4	6	13	6.9	28	87	—	—	—	5, 6
	1.25:1	0.25	10	2.2	10	—	—	—	—	—	—	—	
Methyl α-D-glucopyranoside	2.5:1	0.25	12	1.8	28	—	—	—	—	—	—	—	6
	2.5:1	0.25	10	1.9	24	—	—	—	—	—	—	—	
	5.0:1	0.25	12	2.2	19	—	—	—	—	—	—	—	
D-Glucose	0.25:1	0.03	12	2.3	8	40	6.5	26	60	—	—	—	5
	1.25:1	0.03	12	2.6	10	8	6.8	39	35	9.7	128	57	
	3.75:1	0.03	12	—	—	—	—	—	—	10.7	166	—	
	6.25:1	0.03	12	—	—	—	—	—	—	10.3	218	—	
D-Fructose	0.25:1	0.25	12	2.3	4	80	6.0	25	20	—	—	—	5
	1.25:1	0.25	12	2.3	5	8	6.2	40	67	9.8	—	25	
2-Acetamido-2-deoxy-D-glucose	1.25:1	0.25	12	2.4	11	70	6.6	26	30	—	—	—	5
2-Acetamido-2-deoxy-D-mannose	1.25:1	0.25	12	2.3	10	66	6.3	41	33	—	—	—	5
D-Xylose	0.25:1	0.25	12	2.5	6	76	1.3	—	23	—	—	—	6
	1.25:1	0.25	12	2.2	^{e}	^{e}	1.2	^{e}	^{e}	6.8	^{e}	^{e}	5, 6

[a] The sugar–borate ratio is the molar ratio of sugar to monomeric boron species. [b] δ, chemical shift in p.p.m. downfield of $Me_2O \cdot BF_3$. [c] W, linewidth at half-height (Hz). [d] A, relative area of resonance (%). [e] Values not determined.

Although complete structural assignments for the ^{11}B n.m.r. signals are often impossible, the ring size of the complex and an indication of the stability of the complex can be established.

Glycosides are the simplest carbohydrates to consider for complex formation, since the ring size of the sugar is fixed. The ^{11}B n.m.r. results for methyl α-D-glucopyranoside and methyl α-D-galactopyranoside (Table I) establish the type of complex formed with these carbohydrates. For solutions of methyl α-D-glucopyranoside and borax at pH 12 and 10.5, the resonance observed at δ 1.9 is characteristic of a six-membered borate complex[3]; the ^{11}B n.m.r. signals for borax solutions alone at pH 12 and 10.5 occur at δ 2.4 and 3.3, respectively[4]. This ^{11}B n.m.r. evidence supports Foster's proposed structure[5] (5) for the borate complex of this glycoside in which O-4 and O-6 are bridged by boron. The resonance at δ 1.9 arises from the overlap of the signals from the monocyclic and spirocyclic species. At a higher field strength (28.87 MHz), the signals for monocyclic and spirocyclic species of six-membered ring, borate complexes are resolved[3].



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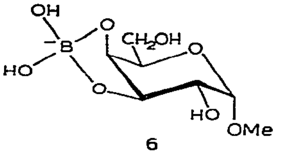

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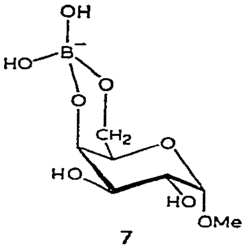

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The configuration of HO-3, HO-4, and HO-6 in methyl α-D-galactopyranoside is such that a 3,4- (6) or a 4,6-complex (7) is possible, and the ^{11}B n.m.r. spectrum shows that both types of complex are formed in solutions at pH 12 and 10 (Table I).

For D-glucose, the ^{11}B n.m.r. results (Table I) show that only a five-membered ring complex is formed, thus excluding the six-membered 2,4-borate complex suggested by Foster[2]. Both monocyclic and spirocyclic complexes co-exist, the latter predominating at high D-glucose–boron ratios. The presence of two bulky molecules of D-glucose surrounding the boron atom in the spirocyclic complex is the likely cause of the very broad resonance of the spirocyclic species (Fig. 1).

The p.m.r. spectrum of D-glucose in solution in aqueous borax at pH 10 (Fig. 2) is considerably different from that of D-glucose alone. For D-glucose, the anomeric signals appear at δ 5.24 (α anomer) and 4.70 (β anomer), whereas, for complexed D-glucose, only one anomeric signal at δ 5.75 is observed. The p.m.r. spectrum also indicates that, at pH 10 in the presence of borax, D-glucose exists exclusively in the complexed form. At pH 7, however, there are signals at δ 5.25 and 4.62, attributable to the anomeric protons of the α and β forms, respectively, of the free sugar, in addition to the anomeric signal of complexed D-glucose at δ 5.75. The pH dependence of the extent of complex formation is clearly shown by these spectra.

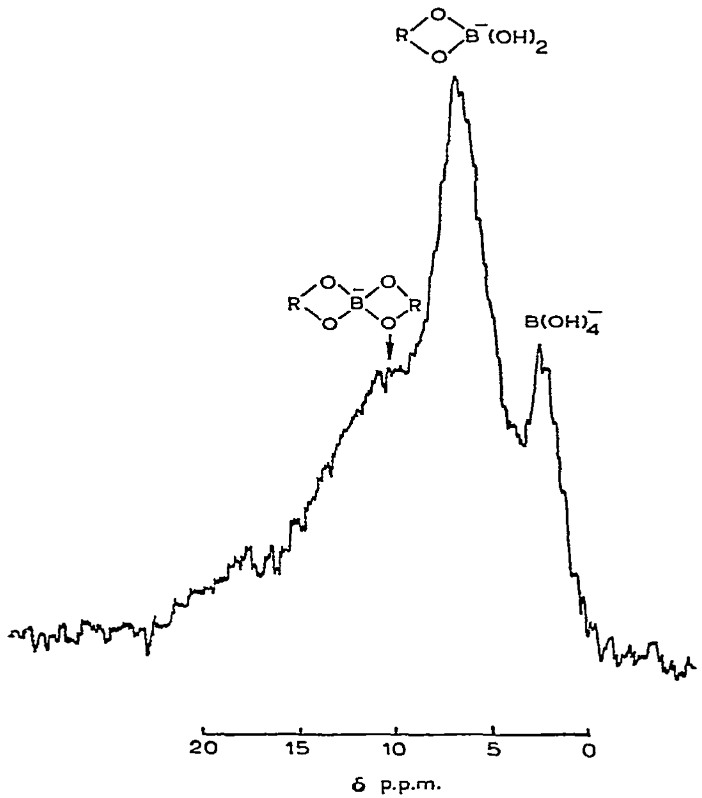


Fig. 1. ^{11}B n.m.r. spectrum of a D-glucose–borax solution (1.25:1 molar ratio) at pH 12; the borax concentration was 50 mg/ml. The reference signal of the methyl ether–boron trifluoride complex at δ 0.0 is not shown.

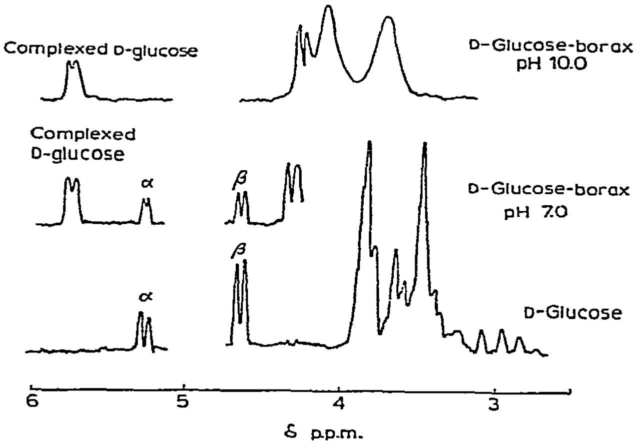
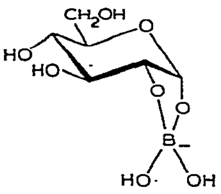


Fig. 2. P.m.r. spectra at 60 MHz of D-glucose (50 mg/ml) in deuterium oxide in the presence and absence of borax (50 mg/ml) at different pH values. DSS is the internal reference at δ 0.0 (the DOH signal at δ ~ 4.8 is omitted).

The p.m.r. spectrum of D-glucose in the presence of borax at pH 10 is the same as that obtained at 40 MHz by Lenz and Heeschen[6], who proposed a 1,2-complex of α-D-glucopyranose (**8**) for the D-glucose–borate complex. Mazurek and Perlin[7] subsequently challenged this assignment, since the p.m.r. spectrum of complexed D-glucose resembles the spectrum of complexed D-glucose derivatives which are incapable of existing in a pyranoid form, *e.g.* the 5,6-carbonate of D-glucose. They proposed a 1,2-complex of α-D-glucofuranose (**9**) from p.m.r. evidence and from thermometric measurements of D-glucose and model furanoid compounds in boric acid solution. The co-existence of both furanoid (**8**) and pyranoid complexes (**9**) in the D-glucose–borax system was not considered, but such a situation is suggested by our p.m.r. study of the D-glucose–benzeneboronic acid system.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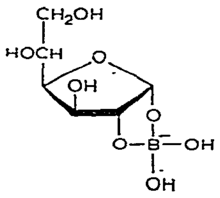
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Complex formation of D-glucose with benzeneboronic acid, as with boric acid, increases with increased pH values (Fig. 3). Comparison of these two situations, however, shows an important spectral difference; two sharp, anomeric doublets at

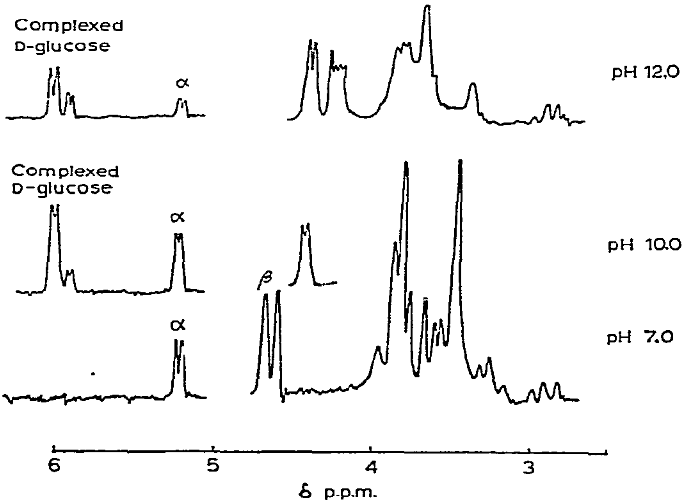

Fig. 3. P.m.r. spectra at 100 MHz of D-glucose (50 mg/ml) in deuterium oxide in the presence of benzeneboronic acid (50 mg/ml) at different pH values with DSS as internal reference at δ 0.0 (the phenyl and DOH signals are omitted).

δ 5.93 and 5.85 arise from D-glucose complexed with benzeneboronic acid, rather than one broad doublet as observed for D-glucose complexed with borate. Thus, two distinct forms of D-glucose, namely the furanoid and pyranoid forms, are complexed with benzeneboronic acid, and the chemical-shift difference between the anomeric signals in these complexes is very small (0.08 p.p.m.). In a similar way, furanoid and pyranoid forms of the borate complex of D-glucose may also co-exist, but in this case the chemical shifts of the anomeric signals of the two forms of D-glucose may be coincident and produce a broad resonance.

The p.m.r. spectrum of D-xylose in the low-field region resembles that of complexed D-glucose. Lenz and Heeschen[6] also observed this similarity, and consequently postulated a 1,2-α-pyranoid complex analogous to that of D-glucose. As in the case of D-glucose, however, it is difficult to make an unequivocal structural assignment from p.m.r. evidence alone, and the ^{11}B n.m.r. results for D-xylose (Table I) suggest other possible structures. The ^{11}B chemical shift of 1.3 indicates the presence of a six-membered ring complex, although, at higher D-xylose–boron ratios, a five-membered ring complex also exists.

Previous work has shown[8] that borate complex formation has a preventive effect on the epimerisation of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-mannose. The ^{11}B n.m.r. data for these compounds in the presence of alkaline borax (Table I) indicate that each sugar forms a complex involving boron in a five-membered ring. It is difficult to postulate a complex which satisfies the ^{11}B n.m.r. results and also explains the effect of borate complex formation on epimerisation. The blocking of epimerisation by eliminating the existence of the aldehydo form would occur if a five-membered ring 1,2-furanoid or -pyranoid complex was formed (*cf.* **8** and **9**), but there is no evidence to support the existence in aqueous solution of a complex involving a boron–nitrogen bond[9].

EXPERIMENTAL

Preparation of samples for ^{11}B n.m.r. studies. — Weighed quantities of borax and sugar were dissolved in distilled water (2.0 ml) to give the required sugar–boron ratio. After equilibration at 33°, the pH of the solutions was adjusted with 10M sodium hydroxide, using a Pye Dynacap pH-meter. The pH adjustments were made at 33°, the temperature of the n.m.r. spectrometer probe, since the pH of these solutions is dependent upon temperature.

Preparation of samples for p.m.r. studies. — Sugars were dried over phosphorus pentaoxide *in vacuo* at 60°. Weighed quantities of the sugar and borax or benzeneboronic acid were dissolved in deuterium oxide and the solutions were freeze-dried. The deuterated compounds thus prepared were dissolved in deuterium oxide with the internal p.m.r. reference, DSS, to give solutions of the required concentrations. The concentrations are indicated on the spectra. The pD of the solution was adjusted to the required value with DCl or NaOD. Since use of the pH-meter probe in the deuterium oxide solutions and the usual methods of pH adjustment would introduce considerable proton–deuterium exchange, the following method of pH adjustment was adopted.

The pH of an analogous solution of sugar, borax, and DSS was adjusted by adding the DCl or NaOD solutions from a micropipette. The volume required for this adjustment was then added to the deuterium oxide system. The pH was reproducible within 0.1 pH unit.

^{11}B n.m.r. spectroscopy. — A Perkin–Elmer R10 spectrometer operating at 12.83 MHz and at a probe temperature of 33.0° was used. Samples were contained in 8.5 mm o.d. tubes and a sample of methyl ether–boron trifluoride ($Me_2O \cdot BF_3$) was used as an external reference. No corrections were made for the difference between the bulk diamagnetic susceptibilities of the sample and the reference.

In most instances, it was necessary to use a computer of average transients (CAT) in order to increase the signal-to-noise ratio. The CAT (Northern Scientific Ltd.) accumulates single spectral sweeps and is triggered automatically by the field sweep of the spectrometer. The sweep rate of each instrument was synchronised.

In favourable circumstances, when signals were well resolved, it was possible to integrate the signals accumulated on the CAT by using the integrating facilities of the spectrometer. A Dupont 310 Curve Resolver was used to obtain the relative areas of the signals for situations in which they overlapped. By synthesising a curve which was coincident with that of the spectrum and switching out each component curve in turn, a measure of the relative area of each signal was obtained.

P.m.r. spectroscopy. — P.m.r. spectra were recorded at 60 or 100 MHz, using Perkin–Elmer R10 or R14 spectrometers, respectively. In each case, the temperature of the probe was 33.0°. Sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) was used as internal reference in the deuterium oxide solutions.

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