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Publisher's version / Version de l'éditeur:
https://doi.org/10.1139/v73-489
Canadian Journal of Chemistry, 51, 19, pp. 3277-3286, 1973-10-01

Carbon-13 Resonance Spectroscopic Studies on the Formation of Borate and Diphenylborinate Complexes of Polyhydroxy Compounds[1]

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Received February 22, 1973

On complexing with sodium tetraborate the ^{13}C magnetic resonance (c.m.r.) spectra of certain sugars contain considerably broadened signals. Replacement of the reagent with sodium diphenylborinate resulted in spectra with sharp singlets corresponding to the diphenylborinate complex, indicating that ^{13}C–^{11}B coupling likely does not occur. Most of the broadening is due rather to either the presence of more than one conformer of the borate complex or the rapid interconversion of the compound with borate complexes of types II and III. In one group of experiments the complexing of diphenylborinate with several low molecular weight sugars and polysaccharides was followed through c.m.r. spectroscopy. In another, complexing was observed with pyranosides having 1,3-hydroxyl groups with orientations that were almost diaxial. In the equilibrium reactions the approximate extent of complexing was estimated and with compounds that can form more than one complex, such as glycerol, the number and structures of the complexes were determined.

Par complexation avec le tétraborate de sodium les spectres de résonance magnétique du ^{13}C de certains sucres comportent un élargissement des signaux considérable. Le remplacement de ce réactif par le diphénylborinate de sodium a pour résultat spectral des singulets fins correspondant au complexe avec le diphénylborinate, montrant que le couplage ^{13}C–^{11}B n'existe probablement pas. La majeure partie de cet élargissement est due plutôt soit à la présence de plus d'un conformère du complexe borate ou à l'interconversion rapide du composé dans les complexes borates de type II et III. Dans une série d'expériences, la complexation du diphénylborinate avec plusieurs sucres de bas poids moléculaire et polysaccharides a été suivie par r.m.n. du ^{13}C. Dans d'autres, cette complexation a été observée avec des pyranosides comportant des groupes hydroxyle-1,3 dont les orientations sont presque diaxiales. Dans les réactions d'équilibration, le pourcentage approximatif de complexation a été évalué; avec des composés pouvant former plus d'un complexe, tel le glycérol, le nombre et les structures des complexes ont été déterminés.
[Traduit par le journal]

Can. J. Chem., 51, 3277 (1973)

Introduction

It was shown in a previous publication (1) that following addition of sodium tetraborate to solutions of carbohydrates in D_2O the formation of borate complexes could be followed by changes in the carbon-13 magnetic resonance (c.m.r.) spectra of the parent compounds. Two types of spectral effects were observed. In one type the signals remain sharp and changes in chemical shift take place and in the other the resonances of the signals remain the same, but broadening of signals of ^{13}C nuclei in the vicinity of the complex occurs[2] (Fig. 3 in ref. 1). Three possible explanations for the latter phenomenon were considered, two of which involve conformational factors. One is that the ring containing the boron atom interconverts rapidly between two or more conformations thus giving an average c.m.r. spectrum. It is more likely that the borate complexes of type II and one or two stereoisomeric type III complexes (Fig. 1) are in equilibrium with the sugar but their interconversion is so fast that they cannot be detected individually by c.m.r. spectroscopy. No change in resonance of the ^{13}C signals was observed since presumably the conformations of the carbon skeletons of sugar and its type II or III complexes were not appreciably different and the carbons were not deshielded to any extent in the borate forms. The other possible explanation is that the signals of ^{13}C nuclei are coupled to ^{11}B nuclei of spin $\frac{3}{2}$ and that quadrupole type broadening occurs.

The latter is now shown to be likely incorrect, however, by the following experiment. In the previous study addition of borax to a D_2O solution of methyl 2,3-di-*O*-methyl-α-D-mannopy-

[1]NRCC No. 13302.
[2]These signals are very stunted and have a line width of 40 Hz. This compares with 5–7 Hz for the other signals. All of the latter were also broadened (to 8–12 Hz) in accord with the finding that a general slight line broadening occurs on addition of sodium diphenylborinate or borate to sugar solutions.

TABLE 1. Chemical shifts of c.m.r. signals of polyhydroxy compounds and diphenylborinate complexes, formed on addition of 1 and 3 mol equiv. of sodium diphenylborinate: estimated percentages of unchanged compounds in the equilibria

Compound and system examined	% of compound in equilibrium with diphenylborinate ester	Relative downfield differences of resonances of signals from that of external TMS in coaxial capillary (δ_C) in p.p.m.																				
1 (a) 1,2:5,6-Di-O-isopropylidene-D-mannitol	—		110.5	—	—	75.8 (C-2)	70.8 (C-3)	—	67.1* (C-1)	38.9	37.3											
(b) 1:1 S-DPB†	11		110.4	78.0	77.3	75.8	70.8	67.5	67.1	39.2	37.8											
2 (a) Methyl 4,6-di-O-methyl-α-D-mannopyranoside	—		101.4 (C-1)	—	—	77.6* (C-4)	—	—	—	71.7 (C-5)	70.8 (C-3)	70.6 (C-4, -6)		—	60.7	—	59.0	55.4				
(b) 1:1 S-DPB	55		101.4	100.9	79.0	77.6	75.4	74.8	71.9	71.7	70.8	70.6		69.0	60.7	60.4	59.0	55.3				
(c) 1:3 S-DPB	26		101.4	100.9	79.0	77.6	75.4	74.8	71.9	71.7	70.8	70.6		69.0	60.7	60.4	59.0	55.3				
3 (a) Methyl 2,3-di-O-methyl-α-D-mannopyranoside	—		—	98.2 (C-1)	80.4 (C-2)	—	77.1 (C-3)	—	73.1* (C-5)	—	—	66.7 (C-4)		61.5 (C-6)	59.0 (OCH_3)	—	57.4 (OCH_3-3)	55.4 (OCH_3-1)				
(b) 1:1 S-DPB	80		98.7	98.2	80.4	79.6	77.1	76.2	73.1	71.6	68.8	66.7	63.4	61.5	59.1	—	57.4	55.5				
(c) 1:3 S-DPB	36		98.7	98.2	80.4	79.6	77.1	76.2	73.1	71.6	68.8	66.7	63.4	61.5	59.1	—	57.3	55.5				
4 (a) myo-Inositol	—		—	75.0	—	—	—	73.0	72.8	71.7*												
(b) 1:1 ratio of sugar to diphenylborinate	38		75.7	75.0	74.8	74.1	—	73.1	72.8	71.8												
(c) 1:3 S-DPB	25		75.8	75.1	74.8	74.1	73.2	—	72.8	71.8												
5 (a) 1,4,5,6-Tetra-O-methyl-myo-inositol	—		—	84.2*	82.7	82.1	—	80.4	—	—	70.9	68.3	60.5	—	—	—	57.3					
(b) 1:1 S-DPB	38		84.9	84.1	82.7	82.0	—	80.4	76.3	71.4	70.8	68.3	60.5	60.3	60.0	59.0	57.4					
(c) 1:3 S-DPB	28		84.9	84.0	82.6	82.0	81.8	80.4	76.3	71.5	70.8	68.3	60.6	60.3	59.9	—	57.4					
6 (a) 1,2:3,4-Di-O-isopropylidene-L-chiro-inositol	—		110.9	79.2 (C-1)	76.7	—	72.1* (C-3)	27.3	25.1													
(b) 1:1 S-DPB	32		110.9	79.2	76.7	76.4	72.1	27.3	25.1													
(c) 1:3 S-DPB	10		111.2	79.2	76.8	76.5	72.0	27.3	25.0													
7 (a) 1,2-O-isopropylidene-α-D-glucofuranose	—		113.0*	—	—	105.2 (C-1)	—	84.9 (C-2)	80.2 (C-4)	—	74.1 (C-3)	—	68.9 (C-5)	—	64.1 (C-6)	26.2	25.7					
(b) 1:1 S-DPB	9		112.9	112.7	106.4	105.2	85.7	85.1	80.2	74.6	—	71.3	—	64.6	—	26.0	25.6					
(c) 1:3 S-DPB	0		—	112.8	106.4	—	85.7	—	80.2	74.6	—	71.4	—	64.6	—	26.7	26.1					
8 (a) 1,2-O-Isopropylidene-3-O-methyl-α-D-glucofuranose			113.0	105.2 (C-1)	—	83.7 (C-2)	—	81.3 (C-3)	80.0 (C-4)	—	68.7* (C-5)	—	—	64.0 (C-6)	58.0	26.1	25.7					
(b) 1:1 S-DPB	34		112.8	105.2	105.0	83.7	82.6	81.3	80.0	69.9	68.7	66.2		64.1	58.1	26.2	25.9					
(c) 1:3 S-DPB	10		113.0	—	105.0	83.7	82.6	81.4	—	69.9	—	66.2	—	—	58.1	26.2	25.9					
9 (a) 6-O-Isopropyl-1,2-O-isopropylidene-α-D-glucofuranose	—		113.0	—	105.2 (C-1)	—	84.9 (C-2)	—	80.4* (C-4)	—	74.2 (C-3	73.6 or CH.Me_2)	—		—	—	70.2	67.9	26.3	25.8	21.8	
(b) 1:1 S-DPB		113.4	113.0	106.2	105.2	85.5	85.0	82.8	80.4	75.1	74.2	73.7	73.0		71.1	70.7	70.2	68.1	26.8	26.4	26.1	21.8
(c) 1:3 S-DPB	0	113.4	—	106.2	—	85.5	—	82.7	—	75.1	74.3	—	73.0	71.1	70.2	—	—	26.8	26.5	26.2	21.9	
10 (a) Methyl α-D-glucopyranoside		—	99.8* (C-1)	—	73.7 (C-2)	—	—	—	72.2 (C-3 or	71.8 C-5)	70.2 (C-4)	—	—	—	61.2 (C-6)	55.5 (OCH_3)						
(b) 1:1 S-DPB	40	100.3	99.9	75.5	73.7	73.3	—	—	72.2	71.9	70.2	67.9	—	63.3	61.3	55.6						
(c) 1:3 S-DPB	22	100.4	99.9	75.6	73.8	73.4	72.8	—	72.2	71.9	70.3	67.9	—	63.3	61.3	55.8						
11 (a) Methyl α-D-mannopyranoside	—	101.5 (C-1)	—	—	—	73.2 (C-5)	71.2 (C-2)	70.6 (C-3)	—	67.5 (C-4)	—	61.6 (C-6)	55.4 (OCH_3)									
(b) 1:1 S-DPB	30	101.5	101.0	76.4	75.0	73.2 71.6	71.3	70.6	69.7	67.5	62.0	61.6	55.5									
12 (a) Methyl β-D-glucopyranoside	—	—	103.8 (C-1)	76.4 (C-3 and C-5)	—	—	—	73.7 (C-2)	—	70.2* (C-4)	—	61.5 (C-6)	57.7 (OCH_3)									
(b) 1:1 S-DPB	30	104.2	103.8	76.5	75.9	75.3	74.5	73.8	71.6	70.3	63.1	61.4	57.8									
13 (a) Methyl 3,6-anhydro-α-D-glucopyranoside	—	—	99.5	—	76.4	—	72.0	71.8	—	70.4	69.8	—	58.5									
(b) 1:1 S-DPB	70	101.6	99.5	77.4	76.4	72.4	72.0	71.8	71.2	70.4	69.8	69.6	58.5									
(c) 1:3 S-DPB	30	101.6	99.6	77.4	76.8	72.4	71.9	—	71.3	70.4	69.8	—	58.5									
(d) 4:1 S-B†	50	100.7	99.5	76.9	76.4	71.8	71.0	70.6	70.0	69.0	58.3											
(e) 2:1 S-B	0	100.7	—	76.9	—	—	71.2	70.8	70.0	69.1	58.3											
14 (a) Methyl 3,6-anhydro-β-D-glucopyranoside	—	—	104.1	—	75.3	72.8	72.5	71.8	—	—	70.2	56.5										
(b) 1:1 S-DPB	45	106.1	104.2	77.6	75.4	72.8	72.4	71.8	71.4	71.0	70.4	56.5										
(c) 1:3 S-DPB	10	106.2	—	77.6	—	—	—	—	71.5	71.2	70.6	56.5										
(d) 4:1 S-B	0	104.5	76.5	71.4	70.4	70.0	56.5															
(e) 2:1 S-B	0	104.7	76.6	71.4	70.4	69.8	56.5															
15 1,6-Anhydro-β-D-glucopyranose	—	102.6	77.4	73.7	72.0	71.4	66.4	31.5														
16 2,4-Di-O-methyl-α,β-L-rhannose	—	94.8	91.7	83.9	83.5	82.9	82.1	74.0	72.2	70.6	68.3	63.0	61.2	59.8	18.2							
17 (a) 1,2-O-Isopropylidene-6-O-methyl-α-D-glucofuranose	—	113.1 (CO)	—	—	105.2 (C-1)	—	88.9 (C-2)	—	80.4* (C-4)	—	—	74.6 (C-3 and	74.0 -6)	—	67.3 (C-5)	59.2	—	—	—			
															26.2	—	25.7					
(b) 1:1 S-DPB	50	113.1	112.9	106.3	105.2	85.4	88.9	83.2	80.4	75.7	75.5	74.6	74.0	70.3	67.3	59.2	58.9	26.2	26.0	25.8		

*Percentage of unchanged compound was estimated by comparison of this signal with that of the MeOH standard.
†S-DPB = ratio of sugar to diphenylborinate and S-B = molar ratio of sugar to sodium tetraborate.

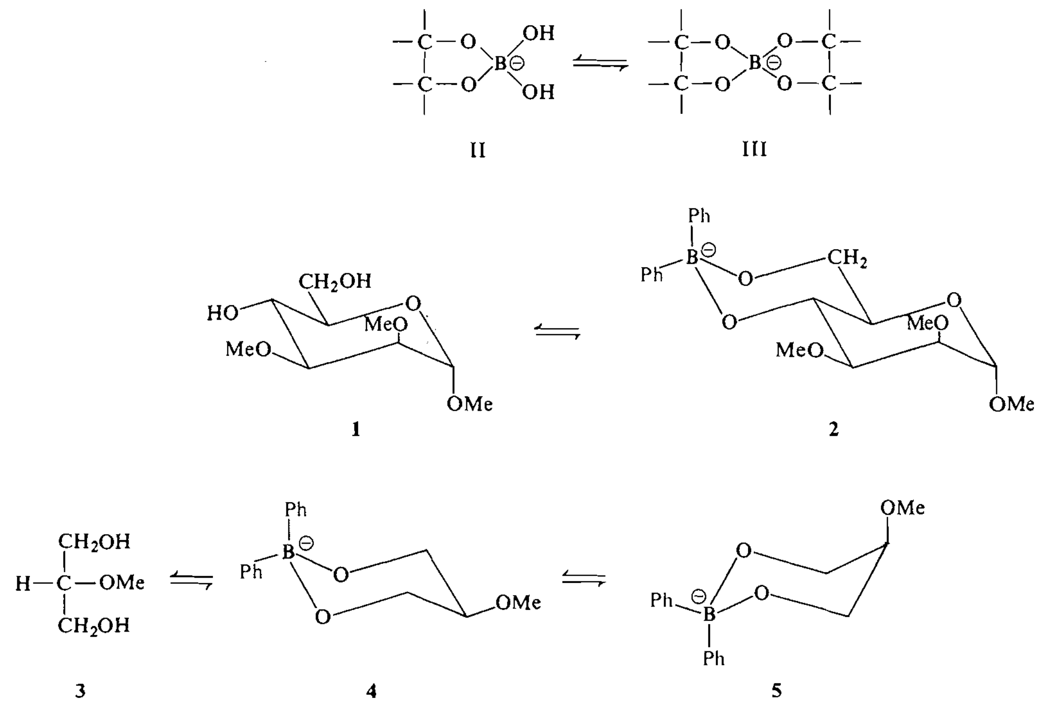


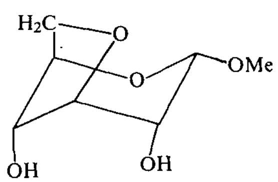
FIG. 1. Equilibria of polyhydroxy compounds with borates and diphenylborinates.

ranoside (Fig. 1; **1**) resulted in marked broadening of the C-4, -5, and -6 signals of the c.m.r. spectrum (Fig. 3 in ref. 1). However, when borax was replaced with 3 mol equiv. of sodium diphenylborinate six new signals appeared (Fig. 3, compare spectra A and B) which corresponded to C-1 to -6 of the diphenylborinate complex (Fig. 1; **2**). No ^{13}C–^{11}B splitting was observed in the ^{13}C signals of carbons in the sugar ring or the aromatic rings of the complex. Their line widths were comparable with those of unreacted sugar.

Two other compounds, *myo*-inositol and 1,4,5,6-tetra-*O*-methyl-*myo*-inositol both gave c.m.r. spectra which contained broadened signals following addition of sodium tetraborate (1). They also complexed with diphenylborinate as indicated by the appearance of new c.m.r. signals (Table 1). This finding is in accord with that of Garegg and Lindström (2) who showed that the p.m.r. spectrum of *epi*-inositol was modified on addition of sodium diphenylborinate. The cyclitol contains several *cis*-1,2-diol groups capable of complexing in addition to 1,3-diaxial hydroxyls which may also possibly react (see later discussion). However, *cis*-cyclohexane-1,2-diol does not form a diphenylborinate complex in appreciable amount since the c.m.r. spectrum remains unchanged on addition of the reagent. The glycol group evidently lacks the inductive effect of adjacent hydroxyls which would favor complex formation. Such an explanation was used by Angyal and McHugh (3) when it was found that on paper electrophoresis in aqueous borax *cis*-cyclohexane-1,2-diol is less mobile than a structurally related compound having an additional *trans*-hydroxyl adjacent to the glycol group.

The degree of complexing of polyhydroxy compounds with diphenylborinate in deuterium oxide cannot be estimated directly on the basis of the signal size of carbons in the complex because of the possibly different nuclear Overhauser effect and the difference of relaxation times of the various nuclei. However, the proportion of uncomplexed material can be estimated by comparing the size of a suitable signal with that of the methyl resonance of methanol (δ_C 49.5), which is used as an internal standard during graded addition of sodium diphenylborinate. Since there are insufficient data points to completely define any given signal the accuracy of the measurements is not great and the percentages quoted for uncomplexed material should be regarded as accurate to only $\pm 10\%$. The chemical shifts of certain polyhydroxy compounds, those of the diphenylborinate compounds, and the percentage of complex formed on addition of 1 and 3 mol equiv. of sodium diphenylborinate are recorded in Table 1.

Many of the diphenylborinate complexes known to be formed on the basis of c.m.r. spectroscopy were previously found to give borate complexes (ref. 1 and references contained therein) and should have structures analogous to the type II (Fig. 1) complex. Compounds containing only two hydroxyl groups with suitable stereochemistry complex on these positions and hexopyranosides should give rise to a 4,6-complex (4). C.m.r. spectral evidence was obtained for complexing of the OH-2 and OH-4 hydroxyl groups of methyl 3,6-anhydro-α-D-glucopyranoside (**6**, Fig. 2) with sodium diphenylborinate since five new c.m.r. signals were obtained (Table 1). With 1 and 3 mol equiv. of reagent 30% and 70% of the complex was formed, respectively. The pyranoid ring of **6** should be in the 1C conformation or a close approximation to it by analogy with 3,6-anhydro-α,α-trehalose hexaacetate (5) and 3,6:3′,6′-dianhydro-α,α-trehalose tetraacetate (6), which had their bicyclic portions assigned this conformation on the basis of p.m.r. spectral evidence. Furthermore, the H-1 signal of **6** had J = 3 Hz, which is very close to the splitting of the H-1 signals of the bicyclic portions of the above derivatives. Examination of Büchi molecular models of **6** show that the restriction of a 3,6-anhydro group distorts the 1C conformation so that the *syn*-axial oxygen atoms on C-2 and -4 are closer than in an unstrained 1C-pyranoid ring.



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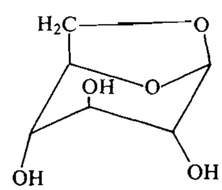

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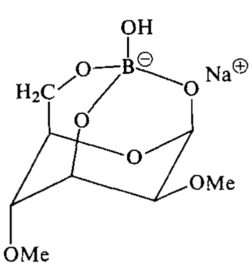

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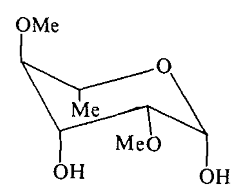

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FIG. 2. Compounds connected with the reaction of borate with 1,3-*syn*-hydroxyl groups.

The β-anomer of methyl 3,6-anhydro-D-glucopyranoside reacts to a greater extent with sodium diphenylborinate (Table 1). In this case p.m.r. spectral examination showed that, in contrast to the α-anomer, the H-1 signals of the free sugar had different chemical shifts. Quantitation showed that 55% and 90% of the complex was formed on addition of 1 and 3 mol equiv. of reagent, respectively. The coupling constants of H-1 of the anhydride and its complex were both 0 Hz, indicating that the dihedral angles of H-1 and -2 of each compound are similar and that the conformation of the anhydride is close to that of its complex, which can only exist in a slightly distorted 1C form. Moreover, the other possibility, that a conformation of the anhydride is a skew-boat form is precluded since the dihedral angle of H-1 and -2 would approach 180°. This would give rise to a large H-1 coupling constant since values of 8–11 Hz are obtained when two vicinal hydrogen atoms on a six-membered ring are *trans* (7). As noted above β-anomer complexes to a higher extent than the α-anomer. This is probably due to non-bonded interactions in the latter between one of the phenyl groups on the borinate ring (in a chair form) and the equatorial OCH_3-1 group.

In a related anhydride, 1,6-anhydro-β-D-glucopyranose (**7**, Fig. 2), c.m.r. spectral examination showed that it does not form a diphenylborinate complex. This is perhaps not surprising since X-ray crystal-structure analysis showed that the *syn*-axial oxygen atoms on C-2 and -4 are separated by 3.30 Å whereas in an unstrained pyranoid system they would be ~2.5 Å apart (8). Although Garegg and Lindström (2) suggested that this type of complexing is possible in *epi*-inositol, which is unstrained and contains 1,3-diaxial hydroxyl groups, the p.m.r. spectrum of the mixed diphenylborinates could not be completely rationalized. It would be of interest to investigate compounds such as this, which can only form complexes between the 1,3-diaxial

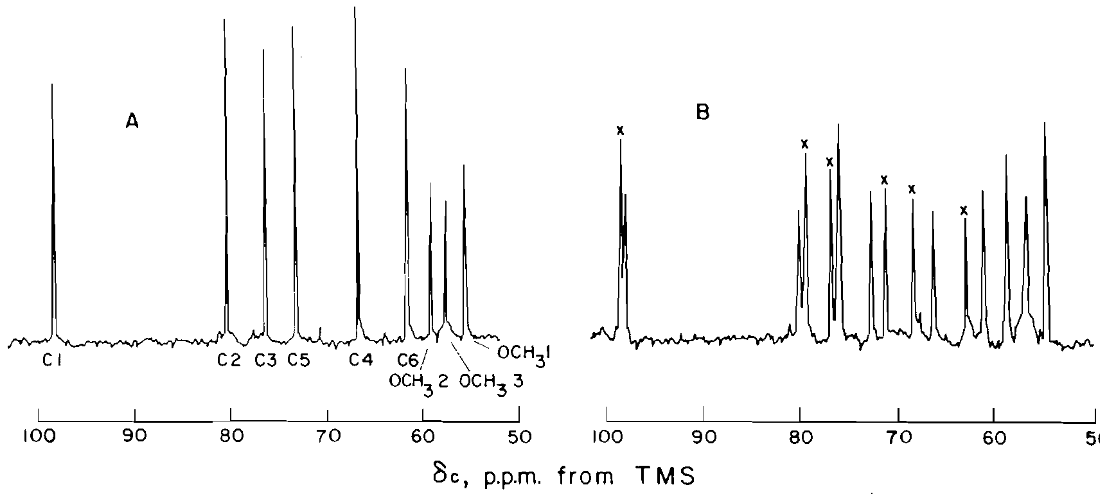

FIG. 3. C.m.r. spectra of methyl 2,3-di-*O*-methyl α-D-mannopyranoside (A) and the equilibrium mixture formed on addition of 3 mol equiv. of sodium diphenylborinate (B) (the new signals of the complex are marked with a cross).

hydroxyl groups. Related compounds, as represented by the 2,4-benzeneboronate of methyl α-D-xylopyranoside (9) and *N*-(*p*-bromophenyl)-α-D-ribopyranosylamine (10), have been prepared, but under kinetically controlled conditions.

The experiments with the anhydrides were parallelled using sodium tetraborate as reagent. C.m.r. spectroscopy showed that whereas the α- and β-anomers[3] of methyl 3,6-anhydro-D-glucopyranoside complex readily, 1,6-anhydro-β-D-glucopyranose does not. On addition of 0.25 mol equiv. of sodium tetraborate (0.5 mol equiv. of "available borate") to methyl 3,6-anhydro-α-D-glucopyranose in D_2O the c.m.r. spectrum showed that approximately 50% of the sugar did not react whereas when the amount of reagent was increased to 0.5 mol equiv. unchanged sugar could not be detected (Table 1). This is suggestive of formation of a type II complex (Fig. 1) in which the ratio of sugar to boron is 1:1. This contrasts with the reaction of tetraborate with methyl 3,6-anhydro-β-D-glucopyranoside since the sugar reacted completely after addition of 0.25 mol equiv. of reagent (Table 1) thus indicating formation of a type III spirane complex (Fig. 1). The c.m.r. spectrum was not altered on addition of a further 0.25 mol equiv. of sodium tetraborate. The difference between the type of borate complex formed suggests that the distance between the *syn*-O-2 and -4 atoms in the α-anomer differs from that of the β-anomer, and that the conformations of the 1C rings are therefore somewhat different.

In a previous publication (1) it was shown by c.m.r. spectroscopy that 2,4-di-*O*-methyl-D-mannose reacts in D_2O with sodium tetraborate to form a borate and a tridentate structure involving O-1, -3, and -6 (**8**, Fig. 2) was proposed. In order to show whether O-6 had a role in complex formation the action of sodium tetraborate and diphenylborinate on 2,4-di-*O*-methyl-α,β-L-rhamnose was determined. Participation was demonstrated since c.m.r. and p.m.r. spectroscopy showed that no reaction occurred with OH-1 and -3 alone in the $1C_\beta$ form (**9**, Fig. 2).

Certain polyhydroxy compounds have a stereochemistry suitable for formation of more than one diphenylborinate complex and c.m.r. spectroscopy can be used for analysis of the mixtures. For example, the c.m.r. spectrum of glycerol in D_2O (Fig. 4, spectrum C) is modified on addition of 3 mol equiv. of sodium diphenylborinate to spectrum F (Fig. 3), which can be rationalized in terms of complexes with five- and six-membered rings. The more prominent features of spectrum F are a broad complex signal centered on δ_C 65.4 and a sharp signal at δ_C 74.2, the latter being assigned to C-2 since it was absent from the c.m.r. spectrum of a solution of DL-glycerol-2-*d* containing diphenylborinate in D_2O. The signal was shown to arise from C-2 of a glycerol 1,2-diphenylborinate anion by consideration of the spectra obtained from 1-*O*-

[3]Electrophoretic mobility of the borate complex of the β-anomer was noted by Theander (34).

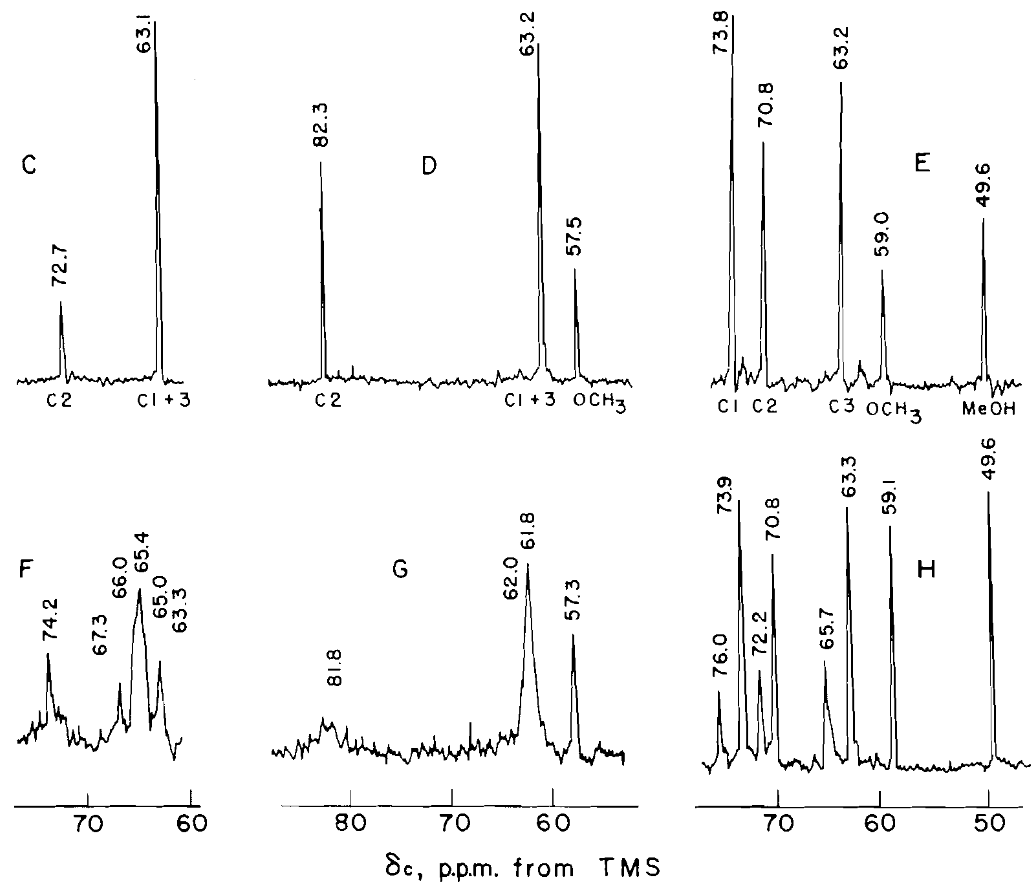

FIG. 4. C.m.r. spectra of glycerol (C) (with 3 mol/mol of sodium diphenylborinate (F)), 2-*O*-methylglycerol (D) (with 1 mol/mol of sodium diphenylborinate (G)), and 1-*O*-methyl-D-glycerol (E) (with 1 mol/mol of sodium diphenylborinate).

methyl-D-glycerol. The spectrum of the polyol contains c.m.r. signals (Fig. 4, spectrum E) which can be assigned as δ_C 73.8 (C-1), δ_C 70.8 (C-2), δ_C 63.2 (C-3), δ_C 59.0 (OCH_3) by taking into account the downfield shift of a CHOH group of a compound when it is converted to a $CHOCH_3$ group (11). Following addition of 1 mol equiv. of sodium diphenylborinate the signals of C-1, -3, and -2 apparently undergo downfield displacements of 2.1, 2.4, and 1.4 p.p.m., respectively (Fig. 4, spectrum H), the last value being close to that of 1.5 p.p.m. observed for the downfield shift of the C-2 signal of glycerol on complexing (Fig. 4; compare spectra C and F). The remaining broad signal centered at δ_C 65.4 (Fig. 4, spectrum F) of the glycerol–diphenylborinate system can be assigned to a 1,3-complex by comparison with the c.m.r. spectrum of the 1,3-diphenylborinate complex of 2-*O*-methylglycerol in D_2O. When an equivalent proportion of reagent was added the C-1 + C-3 signal in spectrum D (Fig. 4) was broadened and shifted downfield by about 1.6 p.p.m. and the C-2 signal was broadened to a line width of 2.8 p.p.m. (Fig. 4, spectrum G). This likely is due to a time averaging effect caused by rapid interconversion of conformers **4** and **5** (Fig. 1). The c.m.r. spectra D and G show that in the 2-*O*-methylglycerol system the equilibrium greatly favors the phenylborinate complex, whereas in the 1-*O*-methylglycerol system the complex is the minor component. This suggests that the diphenylborinate complexes of glycerol are mainly in the six-membered ring form.

Although 1,2-*O*-isopropylidene-α-D-glucofuranose should be able to form two diphenylborinate complexes, it was found that on addition of excess sodium diphenylborinate the c.m.r. spectrum contained only nine signals, which corresponded to formation of only one complex. The

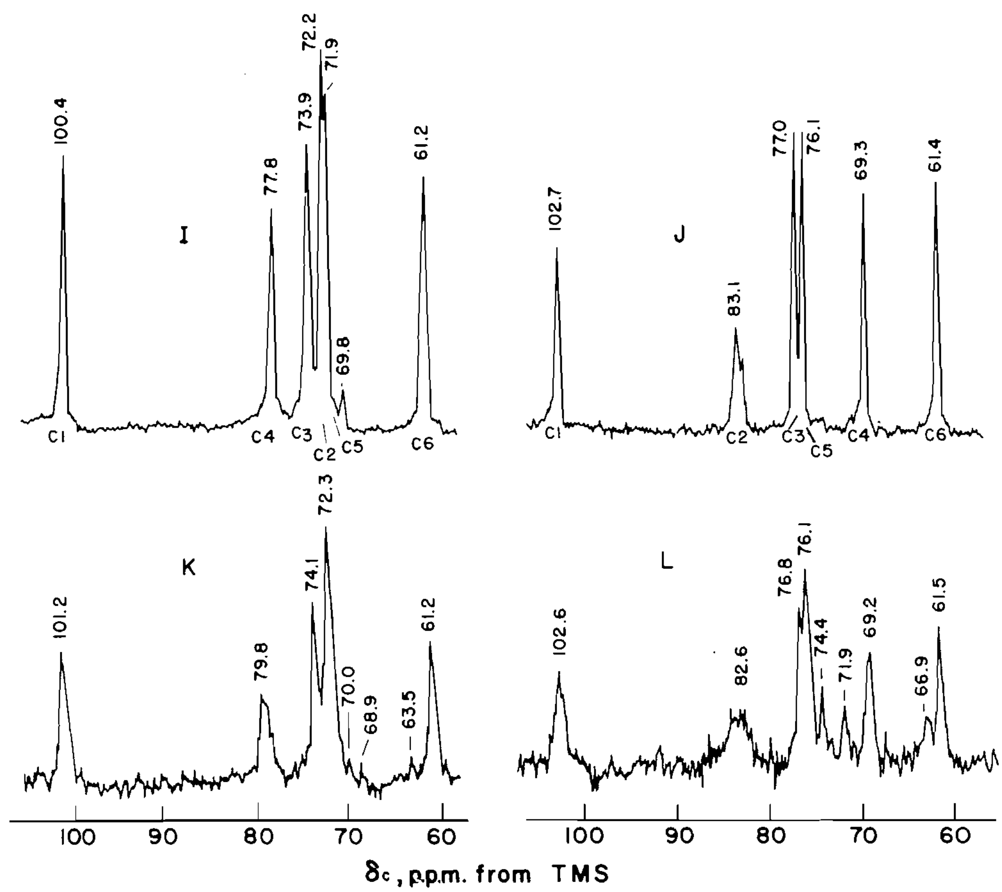

FIG. 5. C.m.r. spectra of soluble potato starch (I) (with 1 mol/mol of sodium diphenylborinate (K)) and a (1→2)-linked β-D-glucopyranan (J) (with mol equiv. of sodium diphenylborinate (L)). The minor signal in *I* is absent in the spectrum of amylose.

equilibrium was on the side of the complex since the new signals greatly predominated in the spectrum following addition of 1 mol equiv. of reagent. In order to determine whether a 5,6- or 3,5-complex was formed the degree of complexing of model compounds was determined following addition of 1 mol equiv. of reagent. Unfortunately 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose on one hand and the 6-*O*-methyl and 6-*O*-isopropyl derivatives of 1,2-*O*-isopropylidene-α-D-glucofuranose on the other, complexed to approximately the same degree and an assignment could not be made (Table 1).

C.m.r. spectroscopy of the borate complexes of polysaccharides is impractical by virtue of the general line broadening phenomenon described above and elsewhere (1) and the ability of borate to form intermolecular complexes which are highly viscous in solution. As sodium diphenylborinate should not give rise to either of these effects a number of polysaccharides of known chemical structure were tested.

The spectral properties of potato starch, which contains mainly (1→4)-linked α-D-glucopyranose units and only relatively few non-reducing end-units that can complex with diphenylborinate, were compared with those of a (1→2)-linked β-D-glucopyranan from *Arthrobacter radiobacter* (12), in which each unit has the potential to complex at the 4 and 6 hydroxyls. After 1 mol equiv. of the reagent was added to potato starch in D_2O, all the signals in the c.m.r. spectrum were broadened, likely due to the presence of sodium ion, and two new very small signals at δ_C 68.9 and δ_C 63.5 appeared. Since two new signals with similar chemical shift appeared when diphenylborinate was added to methyl α-D-glucopyranoside it would appear that they form on a non-reducing end-unit carbon (Table 1; Fig. 5; spectra I and K). On addition of sodium di-

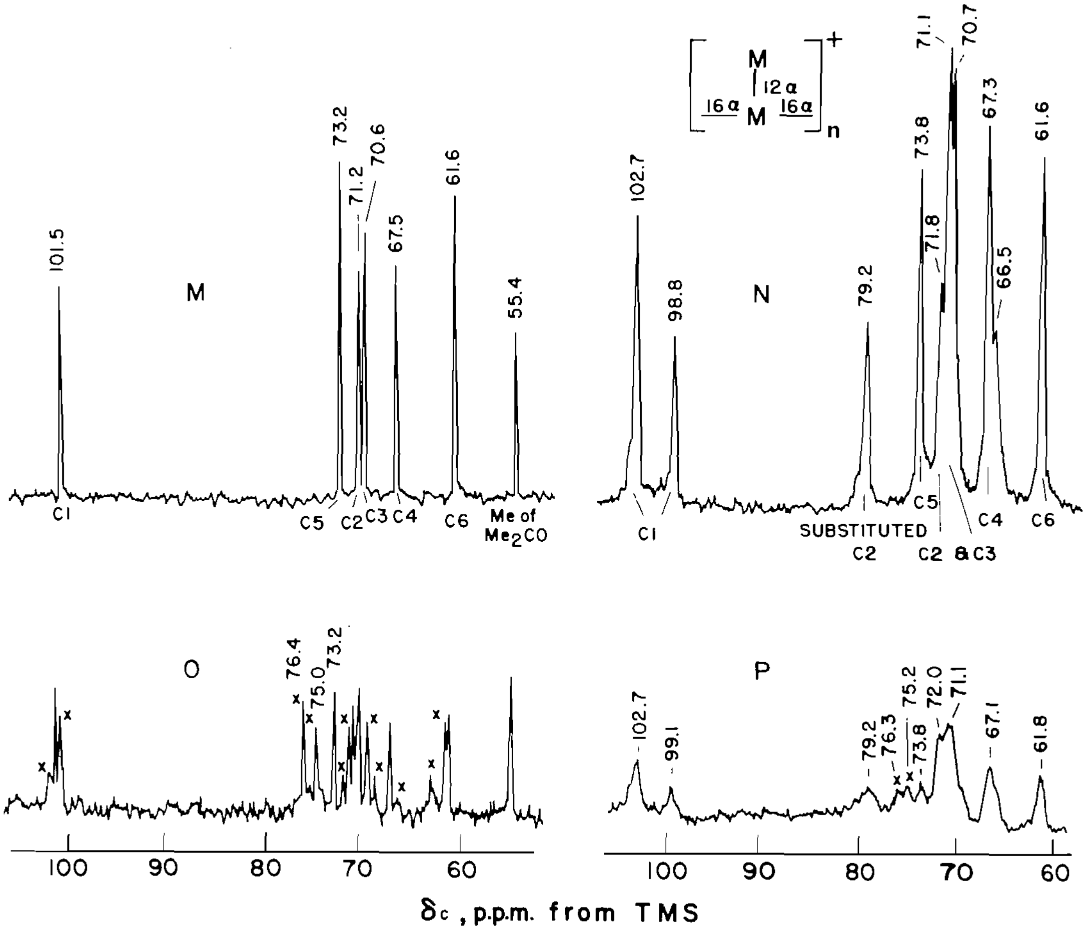

FIG. 6. C.m.r. spectra of methyl-α-D-mannopyranoside (M) and mannan from *E. fibuliger* (N) and those obtained on addition of 1 mol equiv. of sodium diphenylborinate (O and P, respectively) (+structure of mannan; ×signals of complexes).

phenylborinate to a D_2O solution of the (1→2)-linked β-D-glucopyranan the c.m.r. spectrum became less well-resolved and several new signals were formed. In this case two new signals at δ_C 66.9 and δ_C 71.9 appeared, which compares with signals at δ_C 63.1 and δ_C 71.6 in the c.m.r. spectrum of the diphenylborinate of methyl β-D-glucopyranoside. The signal of the glucan diphenylborinate at δ_C 82.6 is much broader than the others.[4] It should arise from C-2 because of its proximity to C-2 in the glucan, which was assigned by its downfield displacement (13) from the C-2 region of β-D-glucose (11). Apparently its resonance is more sensitive to whether the substituted unit contains a diphenylborinate group and whether the glucosyl group at C-2 is diphenylborinated or not.

Interpretation of the c.m.r. spectra of the diphenylborinate derivatives of more complex polysaccharides is more difficult since they contain a larger number of relatively broad signals. However, certain c.m.r. spectra could be partly rationalized. For example, the mannan from *Endomycopsis fibuliger* consists of a (1→6)-linked α-D-mannopyranose main chain with each unit substituted in the 2-position predominantly with single α-D-mannopyranose units (14; see insert in spectrum N, Fig. 6). After addition of 1 mol equiv. of diphenylborinate the c.m.r.

[4]The line widths of the signals of the polysaccharides were broad (20 Hz) compared with those of monosaccharides (5–7 Hz). Even those of starch, which was not complexed to any degree, broadened to about 30 Hz on addition of sodium diphenylborinate.

spectrum developed two new signals at δ_C 76.3 and δ_C 75.2. These could arise from C-2 and -3 of a non-reducing end-unit substituted in the 2- and 3-positions by diphenylborinate, since on complexing the c.m.r. spectrum of methyl α-D-mannopyranoside contained new signals at δ_C 76.4 and δ_C 75.0. When a similar approach was used on the mannans from *Saccharomyces rouxii* (15, 16) and *Saccharomyces cerevisiae* (17), which contain longer side-chains and therefore a lower proportion of non-reducing end-units, comparable new signals of a relatively smaller magnitude were detected. These mannans have (1→6)-linked α-D-mannopyranose main chains with the units substituted in the 2-positions. The mannan from *S. rouxii* contains mainly *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl side-chains and the one from *S. cerevisiae* contains a high proportion of *O*-α-D-mannopyranosyl-(1→3)-*O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl side-chains.

Experimental

Carbon-13 Magnetic Resonance Spectroscopy

C.m.r. spectra were obtained using a Varian XL-100-15 n.m.r. spectrometer with Fourier transform on approximately 1 mmol of carbohydrate in deuterium oxide (2 ml) at 27° contained in a n.m.r. tube (12 mm diameter) incorporating a Teflon plug which prevented vortex formation on spinning. Under these conditions approximately 5000 transients were used. The spectra incorporated 4096 data points when a sweep width of 5000 Hz (198.4 p.p.m.) was used. Quantitative estimation of complexing was carried out by comparison of the size of a given signal with that of the methyl resonance of methanol, used as internal standard. The sweep width was then 2500 Hz (94.2 p.p.m.) and the number of transients correspondingly greater. In the former runs the acquisition time was 0.4 s, the pulse width 50 μs, and the maximum decoupling power (10 W) was used, and was centered on 500 Hz downfield from the ^{1}H resonance of TMS, corresponding to the approximate center of the p.m.r. spectra. The hetero-noise bandwidth was 2000 Hz.

Chemical shifts are in p.p.m., the term δ_C describing the downfield difference of the signal resonance and that of external TMS contained in a coaxial capillary. Under these conditions the methyl signal of methanol is at δ_C 49.5.

Diphenylborinic acid was prepared by the method of Coates and Livingstone (18) from the ethanolamine complex of diphenylboric acid (supplied by Aldrich Chemical Company) and converted to its sodium salt by the procedure of Garegg and Lindström (2). Starting from the complex (5 g) an aqueous solution (20 ml) of the acid was prepared and adjusted to p*H* 10 with aqueous sodium hydroxide. The solution was evaporated, weighed, and then made up to 20 ml in deuterium oxide. In each experiment involving c.m.r. spectroscopy of sugars and their diphenylborinates 1 mmol of polyhydroxy compound was dissolved in deuterium oxide (2 ml) and immediately after preparation of the spectrum 1 mol equiv. of sodium diphenylborinate in deuterium oxide (1.1 ml) was added, the spectra determined, and then a third spectrum was obtained following addition of a further 2 mol equiv. of reagent in deuterium oxide (2.2 ml). The percentage of complexing was estimated by comparing the size of unchanged material with the CH_3 resonance of methanol.

The compounds tested for diphenylborinate complex formation are as follows: 1,2:5,6-di-*O*-isopropylidene-D-mannitol (19), methyl 4,6-di-*O*-methyl-α-D-mannopyranoside (20), methyl 2,3-di-*O*-methyl-α-D-mannopyranoside (21), 1,4,5,6-tetra-*O*-methyl-*myo*-inositol (22), 1,2:3,4-di-*O*-isopropylidene-L-*chiro*-inositol (23), 1,2-*O*-isopropylidene-α-D-glucofuranose (24), 6-*O*-isopropyl-1,2-*O*-isopropylidene-α-D-glucofuranose (25), 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (26), 2-*O*-methylglycerol (27), methyl 3,6-anhydro-α-D-glucopyranoside (28), *S. cerevisiae* mannan (17), *S. rouxii* mannan (15, 16), *E. fibuliger* mannan (14), *A. radiobacter* glucan (12), glycerol (Nichols Chemical Co.), methyl α-D-glucopyranoside (Calbiochem), methyl α-D-mannopyranoside (Sigma Chemical Co.), soluble potato starch (Fischer Scientific Co.), *cis*-cyclohexane-1,2-diol (K. and K. Laboratories Inc.), ethylene glycol (Fischer Scientific Co.), and *myo*-inositol (H. M. Chemical Co.).

1-*O*-Methyl-D-glycerol was prepared by methylation of 1,2-*O*-isopropylidene-L-glycerol (29) with silver oxide – methyl iodide followed by hydrolysis of the product with 80% acetic acid at 100°.

Glycerol-2-*d* was obtained by the reduction of dihydroxyacetone with sodium borodeuteride in deuterium oxide.

1,2-*O*-isopropylidene-6-*O*-methyl-α-D-glucofuranose was obtained by methylation of 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose (30) with silver oxide – methyl iodide followed by removal of the *O*-benzylidene group with 80% acetic acid at 35° for 18 h.

Proton Magnetic Resonance Spectroscopy

The p.m.r. spectra of compounds were obtained using an XL-100-15 Varian n.m.r. spectrometer on a continuous wave mode on the same D_2O solutions as used above. The temperature was 27° and chemical shifts are expressed in δ (p.p.m.) based on the CH_3 resonance of tetramethylsilane contained in a coaxial capillary.

The p.m.r. (and c.m.r.) spectra of 1,6-anhydro-β-D-glucopyranose (31) and 2,4-di-*O*-methyl-L-rhamnose (32) were not changed on addition of sodium tetraborate in one series of experiments and sodium diphenylborinate in another. The percentage of complex formed on addition of sodium diphenylborinate to methyl 3,6-anhydro-β-D-glucopyranoside (33) was measured by comparison of the size of the H-1 signal of the sugar at δ 5.30 (J = 0 Hz) with that of H-1 of the complex at δ 5.37 (J = 0 Hz). The H-1 signal of methyl 3,6-anhydro-α-D-glucopyranoside was at δ 5.47 (J = 3 Hz) and was indistinguishable from that of its diphenylborinate.

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