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^{13}C MAGNETIC RESONANCE SPECTROSCOPIC EVIDENCE FOR FORMATION OF BORATE COMPLEXES OF POLYHYDROXY COMPOUNDS*

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ABSTRACT

Borate complexes, formed on addition of sodium tetraborate to solutions of carbohydrates in D_2O, can be detected by monitoring changes in the ^{13}C magnetic resonance spectra of the parent compounds In one type of change the chemical shifts remain constant, but broadening of signals of ^{13}C atoms in the vicinity of the complex occurs In the other type, the signals remain sharp, but changes in chemical shift take place In addition to permitting the detection of borate complexing, it is often possible to ascertain the chemical structures present and to determine quantitatively the relative proportions of complexes and starting materials A wide variety of polyhydroxy compounds was examined and the formation of complexes of types II, III, and IV assessed Only two of the 19 compounds examined, 1,2 5,6-di-*O*-isopropylidene-D-mannitol and *cis*-inositol, undergo spectral changes on addition of boric acid because of formation of type I and type IV complexes, respectively

INTRODUCTION

The cyclic borate complexes of carbohydrates that are formed in solution by the action of tetraborate or boric acid have been studied extensively Although they can exist in many forms (Fig 1), as weakly ionised esters (type I) formed from boric acid, or as anions of the types II, III, and IV formed from tetraborate, the strict requirements for their formation have made them useful in studying problems of structure and stereochemistry They can be detected as borate esters (Type I) by conductivity measurements[1], as anionic complexes, types II, III, and IV, by zone electrophoresis[2,3,4] in tetraborate solution, as a type IV complex by the pH decrease observed on addition of carbohydrate to tetraborate solution[2,5], or by specific-rotational measurements[6] Mazurek and Perlin[7] have studied the borates formed from five-membered vicinal diols by thermometric measurement of vapor-pressure equilibria and by p m r spectroscopy of tetraborate solutions

The detection of complex formation by p m r. spectroscopy can be used to determine the position(s) of substitution in certain α-D-mannopyranose derivatives[8]

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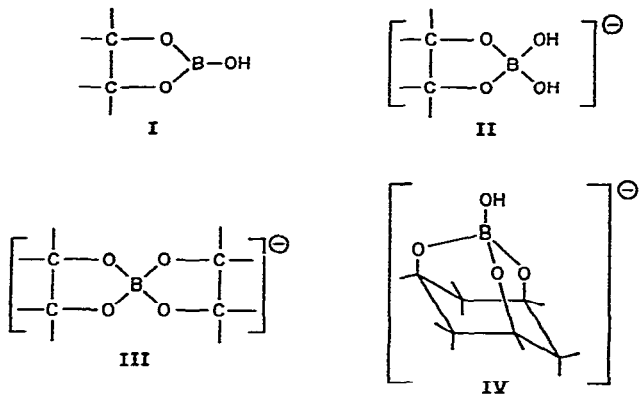

Fig 1 Types of borate complexes

Those containing free *cis*-2,3-diols react with excess tetraborate forming 2,3-borate complexes, which cause a downfield shift of the H-1 p m r. signal of the mannoside by 10–14 Hz This contrasts with a shift of 2–5 Hz occurring with 2- or 3-substituted mannosides The present study is an expansion of the foregoing approach by using ^{13}C magnetic resonance (c m r) spectroscopy, a technique that gives a less crowded spectrum, when the proton noise-decoupling technique is used

RESULTS AND DISCUSSION

Initial c m r studies were conducted to examine the effect of adding sodium tetraborate on the ^{13}C spectra of various α-D-mannopyranoside derivatives dissolved in deuterium oxide The derivatives were selected because of the possibility of borate complexing at the 2,3- or 4,6-positions, or at both Sodium tetraborate was generally added progressively to the solutions so that the ratio of sugar to "available borate", was 2 1, 1 1, and 1 2 The available borate is calculated from the equation

$$Na_2B_4O_7 + 7H_2O = 2Na^+ + 2B(OH)_4^- + 2H_3BO_3$$

This means, for example, that this ratio is 2 1 when the molar ratio of sugar to $Na_2B_4O_7$ is 4 1

In order to aid interpretation of the effect of tetraborate on the c m r spectra of methyl *O*-methyl-α-D-mannopyranosides, assignments of the signals were made This was greatly facilitated by the observation by several workers on inositols[9] and aldopyranoside derivatives[10,11] that the resonance of a ^{13}C atom is shifted considerably downfield on methylation of the attached hydroxyl group Our assignments which are made on this basis, are summarized in Fig 2 The assignments for methyl α-D-mannopyranoside agree with those of Dorman and Roberts[10] and Perlin *et al* [11]

1 Borates of methyl O-methyl-α-D-mannopyranosides — Marked effects on the c m r spectra of methyl 4,6-*O*-ethylidene-α-D-mannopyranoside and methyl

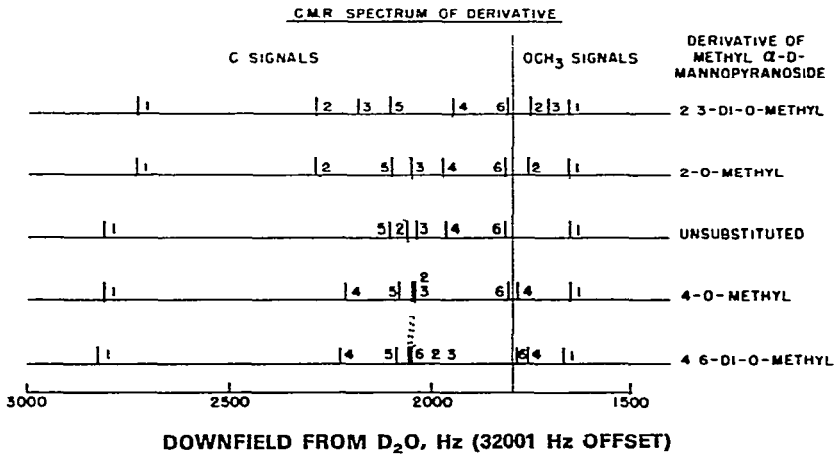

Fig 2 Assignments of ^{13}C signals of methyl α-D-mannopyranoside and its *O*-methyl derivatives, based on downfield shifts of ^{13}C signals on methylation of their adjacent hydroxyl groups

4-*O*-methyl-α-D-mannopyranoside in D_2O occurred on addition of sodium tetraborate A number of the signals of the 4,6-*O*-ethylidene derivative were almost completely replaced with new ones of different chemical shift when a ratio of sugar to available borate of 1 2 was reached The resulting spectrum (Table I) indicated the existence of a preponderating borate complex (type II) having a sugar to borate ratio of 1 1, as it displayed 10 signals, close to the 9 expected for a compound of this type Increasing the ratio to 1 4 gave a spectrum having 9 signals

At a sugar to available borate ratio of 2 1; sixteen c m r signals were obtained, some of them being different from those of the 1 1 complex and the starting material This is explained by the formation of two isomeric 2 1 spirane complexes (type III)

Progressive addition of sodium tetraborate to D_2O solutions of methyl 4-*O*-methyl-α-D-mannopyranoside was conducted up to a sugar to available borate ratio of 1·2 At this level, four new signals were observed (Table I) A new C-1 signal at 2798 Hz appeared, and a pro-rated comparison of the size of the original C-1 signal at 2823 Hz with the unchanged C-6 signal at 2050 Hz showed that one half of the 4-*O*-methyl derivatives was complexed Methyl 4-*O*-methyl-α-D-mannopyranoside therefore appears to have an alignment of the 2- and 3-OH groups somewhat less suitable for borate complexing than that of methyl 4,6-*O*-ethylidene-α-D-mannopyranoside, which forms the 1 1 complex exclusively

A superior, and more generally applicable, method of analysis was devised that involves use of a 2% solution of acetone as an internal standard The diminution of concentration of the polyhydroxy compound on addition of borate was estimated quantitatively by measurement of the decrease in size of the signal intensities of the polyhydroxy compound relative to the methyl resonance of the acetone at 1060 Hz This method, which obviates the unpredictable signal responses of a Fourier transform

TABLE I

C M R -SPECTRAL CHANGES OBSERVED ON ADDITION OF SODIUM TETRABORATE TO D_2O SOLUTIONS OF CARBOHYDRATES CONTAINING *cis*-1,2-DIOL GROUPS IN PYRANOSIDE AND FURANOSIDE RINGS

Compound and system examined	*Relative downfield shifts of signals in Hz, with a sweep offset of 32001 Hz, and a D_2O lock*[a]															
1 (a) Methyl 4,6-*O*-ethylidene-α-D-mannopyranoside	2850	—	2810	—	—	2238	—	—	—	2058	1988	1880	—	—	1668	773
(b) 2 1, 1 1	2850	2820	2810	2300	2295	2238	2140	2083	2075	2058	1988	1878	1810	1805	1668	773
(c) 1 2	—	2820	2808	2303	—	—	2140	—	2073	—	1988	—	1810	1805	1668	775
2 (a) Methyl 4-*O*-methyl-α-D-mannopyranoside	2823 (C-1)	—	—	2220 (C-4)	—	—	2085 (C-5)	2055 (C-2)		2050 (C-3)		1813 (C-6)	1795 (4-OMe)		1663 (1-OMe)	
(b) 2 1, 1 1, 1 2	2823 (C-1)	2798 (C-1)		2268	2220	2158	2138	2085	2055	2030		1815	1795		1663	
3 (a) Methyl 2,3-di-*O*-methyl-α-D-mannopyranoside	2743 (C-1)	2293 (C-2)		2190 (C-3)	2110 (C-5)		1948 (C-4)	1820 (C-6)		1758 (2-OMe)		1715 (3-OMe)	1665 (1-OMe)			
(b) 2 1, 1 2	2748	2290		2190	2210[b]		1930[b]	1825[b]		1758		1713	1668			
4 (a) Methyl 2-*O*-methyl-α-D-mannopyranoside	2743 (C-1)	2295 (C-2)		2110 (C-5)	2055 (C-3)		1975 (C-4)	1820 (C-6)		1765 (2-OMe)		1663 (1-OMe)				
(b) 2 1, 1 2	2748	2298		2115[b]	2058		1978[b]	1822[b]		1768		1665				
5 (a) Erythritan	—	—		—	2083 (C-2,3)		2068 (C-1,4)									
(b) 4 1	2210	—		2165	2083		2068									
(c) 2 1	2210	2170		2165	2083 (tr)[c]		2068 (tr)[c]									
(d) 1 1, 1 2	2210	2170		2165	—		—									
6. (a) Methyl 5,6 di *O*-methyl-α-D-mannofuranoside	—	3010		—	—		2255	2225	2213	—	2073	2058	1763	1730	1695	—
(b) 2 1	—	3015		2330	2318		2275	2228	2223	2195	—	2063	1763	1720	—	1648
(c) 1 1	3020	3015		2328	2318		2275	2226	2223	2193	—	2063	1763	1723	—	1648
(d) 1 2	3020	3015		2330	2318 (tr)[c]		2275	—	2223	2193	—	2063	1765	1723	—	1648

[a]Under these conditions the methyl resonances of acetone is 1060 Hz [b]Signal shortened and broadened on addition of tetraborate Figures in parentheses refer to assignment of carbon or methoxyl [c]tr = trace signal

system, gave the same percentage of complexed 4-*O*-methyl derivative as already recorded

A different spectral effect was observed in experiments on methyl 2-*O*-methyl-α-D-mannopyranoside Although no changes in chemical shift of the c m r signals occurred on the addition of borate, 3 of the 8 signals were simultaneously shortened and broadened to a line width of 40 Hz (Table I) (The possible causes of this phenomenan are discussed in detail at the end of the Results and Discussion section) The effect was most marked with the C-4 and C-5 signals, and was somewhat less with the C-6 signal (for assignments see Fig 2) It appears therefore that the sugar is largely converted into a 4,6-borate complex

A similar effect was noted with methyl 2,3-di-*O*-methyl-α-D-mannopyranoside as, on addition of borate, marked broadening of the C-4 and C-5 signals and some broadening of the C-6 signal occurred (Table I and Fig 3) These observations are reasonably consistent with the findings of Foster and Stacey[12] who examined a number of alkyl glucopyranoside derivatives by paper electrophoresis in the presence of 0 2M sodium tetraborate Those derivatives containing free 4,6-hydroxyl groups gave complexes and had M_G values (rates of migration compared with glucose) of up to 0 20. These values are low, however, and seem to indicate that only small proportions are complexed, since the M_G value is an approximate indication of the proportion of ionic borate complex formed

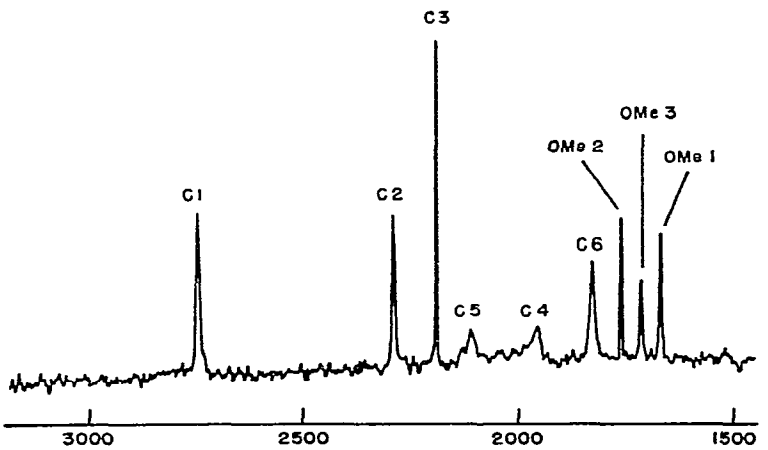

Fig 3 C m r spectrum of methyl 2,3-di-*O*-methyl-α-D-mannopyranoside plus sodium tetraborate in D_2O

The c m r spectrum of methyl α-D-mannopyranoside in the presence of excess sodium tetraborate was very complex Considerable broadening of all signals except for the methoxy signal occurred This observation is consistent with the "broadening" property observed above

Summarizing, these c m r results show that two spectral effects can occur on borate complexing One of them manifests itself by a broadening of ^{13}C signals of carbon atoms that exist close to the borate complex in the molecule, but with no change of chemical shift The other effect is a change in chemical shift of the ^{13}C signals on borate complexing For example, in the case of the c m r spectra of methyl 4,6-*O*-ethylidene-α-D-mannopyranoside and those of its borate complexes, it appeared that two 2 1 and one 1·1 sugar–borate complexes (type II and III) were formed. The c m r method appeared to have potential in analysis of borate complexes in solution, and a systematic examination of other compounds that can form borate complexes was therefore performed

2 Borates of cis-*1,2,-diols in a five-membered ring* — Mazurek and Perlin[7] showed that *cis*-3,4-dihydroxytetrahydrofuran (erythritan) complexes readily with potassium tetraborate When the proportion of diol to available borate ion was 2:1 the degree of complexing, according to thermometric vapor-pressure measurements, was 70% of that expected for formation of a spirane complex (Type III) The c m r spectrum of a similar proportion of erythritan to sodium tetraborate in D_2O indicates a marked tendency to form this complex The spectrum shows two main signals at 2210 and 2165 Hz, the latter having a very small shoulder (<5%) at 2710 Hz (Table I) Two minute signals at 2083 and 2068 Hz, corresponding to unchanged erythritan, were also present These were shown to arise from C-2 and C-3, and C-1 and C-4, respectively, by heteronuclear decoupling experiments with the proton assignments of Mazurek and Perlin[7] as a basis When the ratio of diol to available borate was changed to 1 1, the signal at 2170 Hz increased in size and thus appeared to arise from a complex of type II The signal at 2210 Hz is common to the type II and type III complex By comparison of the 2165 Hz signal with that of an internal acetone standard, the ratio of spirane type III complex to type II complex was found to be 1 to 1 2 A diol to available borate ratio of 1 2 results in a type III to type II complex-ratio of 1 to 2

Sodium tetraborate reacts with methyl 5,6-di-*O*-methyl-α-D-mannofuranoside (**1**) in D_2O, and the reaction is similar to the previous one except that two different stereoisomers of the spirane type III complex can be formed This is shown by the complete disappearance of the mannoside signal at 2073 Hz in a solution having a mannoside to available borate ratio of 2 1 (Table I) As the spectrum shows 11 instead of the 9 signals expected from a single spirane isomer, it appears that two isomeric forms, **2** and **3**, are present When higher levels of borate were used, modification of the spectrum occurred because of the formation of a complex having structure **4** (Table I and Fig 4)

cis-*1,2-Diols in a six-membered cyclitol ring* — Angyal and McHugh[5] have demonstrated that certain cyclohexanediol and cyclohexanetriol derivatives containing *cis*-1,2-diol groups migrate only slowly on paper electrophoresis in sodium tetraborate solution This indication of a low degree of complexing is consistent with the present c m r data The spectrum of *cis*-cyclohexane-1,2-diol in D_2O, showing signals at 2060, 1015 and 813 Hz, is affected by addition of excess sodium tetraborate,

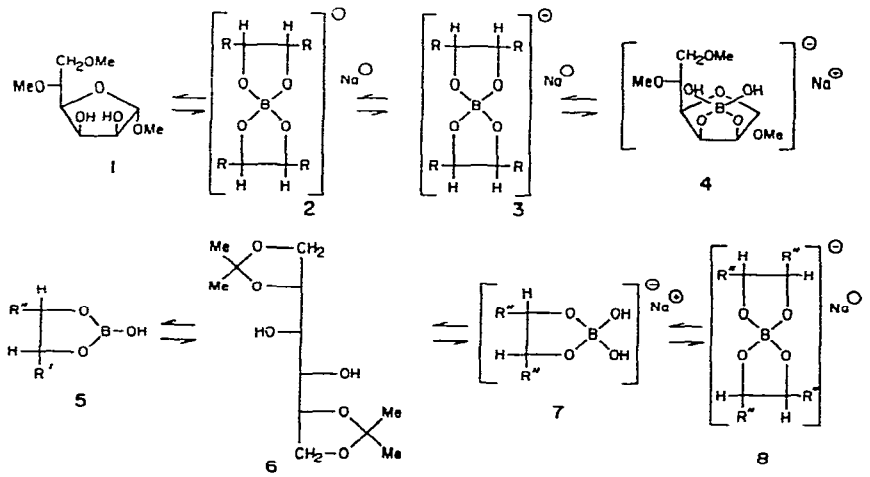

Fig 4 Borate complexes formed with methyl 5,6-di-*O*-methyl-α-D-mannofuranoside and 1,2 5,6-di-*O*-isopropylidene-D-mannitol

in contrast to the behavior with the *trans*-isomer Some broadening and shortening (50% of original size) of the ^{13}CHOH signal at 2060 Hz occurs, the effect being greater than for the signal at 1015 Hz that corresponds to C-3 and C-6 (Table II) By analogy with methyl 2-*O*- and 2,3-di-*O*-methyl-mannopyranoside derivatives, this effect, although less marked, is interpreted as being due to partial borate complexing

Broadening and shortening of signals occurs to some degree on addition of tetraborate to solutions of other cyclitols containing *cis*-1,2-diol groups in a six-membered ring, such as 1,4,5,6-tetra-*O*-methyl-*myo*-inositol and 1,2,3/4-cyclohexanetetrol (Table II)

The low apparent degree of borate complexing indicated by these data contrasts with that observed with methyl 4,6-*O*-ethylidene-α-D-mannopyranoside and methyl 4-*O*-methyl-α-D-mannopyranoside, which contain *cis*-2,3-hydroxyl groups in a six-membered ring It is possible that stabilization of the type II borate complexes occurs, possibly by hydrogen bonding of a borate hydroxyl group with the oxygen atom of the pyranoside ring

With 1,2 3,4-di-*O*-isopropylidene-L-*chiro*-inositol, the available hydroxyl groups, although 1,2-*trans*, are aligned in the strained system somewhat as 1,2-*cis*-hydroxylgroups in an unsubstituted six-membered ring This is indicated by the ready formation of 1,2 3,4 5,6-tri-*O*-isopropylidene-L-*chiro*-inositol[13] Borate complexing is evidenced by c m r spectroscopy, as two of the signals at 2200 and 2085 Hz are broadened considerably (to an extent comparable to that in Fig 3) on addition of excess tetraborate (Table II)

According to successive irradiation experiments with the OH-3 and OH-4 signal of 1,2 3,4-di-*O*-isopropylidene-L-*chiro*-inositol (dimethyl sulfoxide-d_6 as

TABLE II

C M R SPECTRAL CHANGES OBSERVED ON ADDITION OF SODIUM TETRABORATE (OR BORIC ACID) TO D_2O SOLUTIONS OF CARBOHYDRATES CONTAINING 1,2-DIOL GROUPS IN STRAIGHT CHAINS OR SIX-MEMBERED CYCLOHEXANE RINGS

Coumpound and system examined	*Chemical shifts* (see Table I)									
1 *cis*-Cyclohexane-1,2-diol	2060[a]	1015[a]		813						
2 *trans*-Cyclohexane-1,2-diol	2170[a]	1105		883						
3 1,2,3/4-Cyclohexanetetrol	2163[a]	2130[a]		2045	2028	988	925			
4 1,4,5,6-Tetra-*O*-methyl-*myo*-inositol	2388[a]	2350[a]		2335	2293	2053[a]	1988[a]	1973	1788	1713
5 Ethylene glycol	1860									
6 (a) 1,2 5,6-Di-*O*-isopropylidene-D-mannitol	3050				2178	2058(C-3)	1950	935	900	
(b) 2 1 ratio of sugar to available borate	3050	2225	2218	2185(tr)[b]	2178	2058(tr)[b]	1950	938	900	
(c) 1 1 ratio of above	3050	2225	2218	2185	2178		1953	935	900	
(d) 3 1 ratio of boric acid to sugar	3050	2073	2055	2048	1948	1875		930	898	
7. (a) 1,2,3,4 Di-*O*-isopropylidene-L-*chiro*-inositol	3065	2265	2200	2085(C-3)	958	900				
(b) 1 2 ratio of inositol to available borate	3070	2260	2203[a]	2085[a]	955	898				

[a]Signal shortened and broadened on addition of tetraborate [b]tr = trace

solvent) as a basis, assignments were made for the H-3 and H-4 signal and thence to the C-3 and C-4 signal, which had a resonance at 2085 Hz This signal, was together with the signal at 2200 Hz, was broadened on treatment of the compound with borate The latter signal probably arose from the adjacent 2 and 5 carbon atoms, by analogy with the effect on the signal of carbon atoms adjacent to the CHOH groups of *cis*-cyclohexane-1,2-diol

3 Borates of straight-chain 1,2-diols — The c m r spectrum of ethylene glycol in D_2O was unaffected by addition of sodium tetraborate Little borate complexing occurs therefore, probably because of the greater thermodynamic stability of the compound having the hydroxyl groups in the staggered form, in comparison with the borate complex

A compound having hydroxyl groups in a more favorable orientation for borate complexing was selected, namely 1,2 5,6-di-*O*-isopropylidene-D-mannitol

(Fig 4; **6**) This compound is analogous to *threo*-butane-2,3-diol, which migrates in borate solution at 4 times the rate of *erythro*-butane-2,3-diol[14] In D_2O, the mannitol derivative gave a c m r spectrum showing 7 signals, (Table II) The two high-field signals at 900 and 935 Hz arose from the two *O*-isopropylidene C-methyl groups and that at 3050 Hz from the adjoining carbon atom. One other assignment was made by using the p m r spectrum of **6** as a basis One of the signals, which was considerably simplified by changing the solvent from dimethyl sulfoxide-d_6 to D_2O, was evidently that of H-3 which is coupled to the OH-3 group Irradiation at the frequency of H-3 simplified the ^{13}C signal at 2178 Hz, showing the latter to arise from C-3.

Addition of sodium tetraborate to a solution in D_2O of 1,2 5,6-di-*O*-isopropylidene-D-mannitol, in an amount equivalent to a 2 1 ratio of sugar to available borate gave, mainly a 2 1 spirane complex (**8**) according to c m r data (Table II) Examination of molecular models showed that only one isomer is possible Less than 5% of the free sugar, as indicated by the small signal at 2058 Hz, had not reacted Increasing the ratio to 1 1 gave, in addition to the foregoing signals, an appreciable signal at 2185 Hz, which arises from a 1 1 complex (**7**)

Formation of a complex of boric acid with 1,2 5,6-di-*O*-isopropylidene-D-mannitol occurred, as the c m r spectrum was considerably modified after addition of boric acid (Table II) The complex appeared to have structure **5**, as the pattern of the spectrum differed considerably from those of **7** and **8** Of the other compounds cited in the present work, only *cis*-inositol was shown to react to any appreciable extent with boric acid in D_2O

4 Tridentate borate complexes — Angyal and McHugh[2 5] found that, on addition of *cis*-inositol to aqueous solutions of sodium tetraborate, a decrease in pH took place on formation of a tridentate type of borate complex (Fig 5 **10**, R = Na^+)

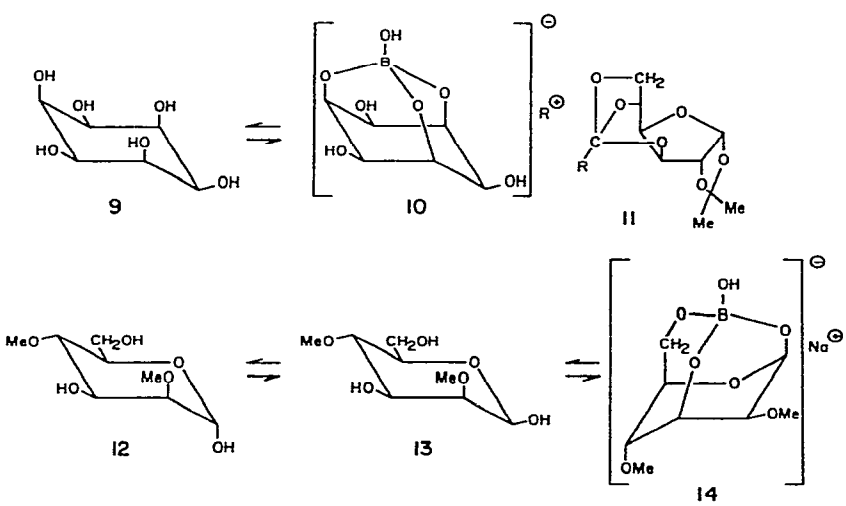

Fig 5 Tridentate complexes formed with *cis*-inositol and 2,4-di-*O*-methyl-D-mannose ortho ester formation with 1,2-*O*-isopropylidene-α-D-glucofuranose

TABLE III

C M.R. SPECTRAL CHARGES OBSERVED ON ADDITION OF SODIUM TETRABORATE OR BORIC ACID TO D_2O SOLUTIONS OF CARBOHYDRATES CAPABLE OF FORMING TRIDENTATE BORATE COMPLEXES

Compound and system examined	*Chemical shifts* (see Table I)								
1 (a) *cis*-Inositol	—	—		2123[a]		2000[a]	—	—	
(b) 2 1-ratio of cyclitol to available borate	2185	—		2123[a]		2000[a]	—	1968	
(c) 1 1 and 1 2 ratio of above	2185	—		—		—	—	1968	
(d)1 3 molar excess of boric acid	—	2140		2123[a]		2000[a]	1975	—	
2 (a) *epi*-Inositol	—	2158		—		2090	2049	—	
(b) 2 1 ratio of cyclitol to available borate	2205	2158		2135		2090	2048	2005	
(c) 1 1 and 1 2 ratios of above	2205	—		2135	—	—	—	2005	
3 (a) *myo*-Inositol	2158	2108		2103		2078			
(b) 2 1 and 1 1 ratio of available borate	2158		2110			2078	2068	2018	1855
4 (a) 2,4-Di-*O*-methyl-D-mannose	2650 (β-C-1)		2568 (α-C-1)			—	2340	2320	2330
(b) 2 1, 1 1 and 1 2 ratios of aldose to available borate	2560		2568			2548	2343	2323	2233
5 (a) 1,2-*O*-Isopropylidene-α-D-glucofuranose	3120	—		2918		—	2408	2290	—
(b) 2 1 ratio of sugar to available borate	3115	2933		2920		2423	2410	2290	2173
(c) 1 1 ratio of above	3113	2933		—		2423	—	2288	2173
(d) 6-*O*-Isopropyl-1,2-*O*-isopropylidene-α-D-glucofuranose	3120	—	2918	—	2408	—	2295	—	2125
(e) 2 1 ratio of sugar to available borate	3118	2935	2918	2423	2410	2315	2295	2160	2123
(f) 1 1 ratio of above	3115	2933	—	2423	—	2313	—	2160	2120

[a]Broad signal with line width of 150 Hz

Their finding that the equilibrium reaction favored the complex almost exclusively was confirmed by c m r spectroscopy In D_2O, *cis*-inositol gives a c m r spectrum showing two signals that are much broader than usual, presumably because of rapid interconversion of one chair form to the other (Table III) Addition of sodium tetraborate in half of the amount necessary to form a 2 1 cyclitol–borate complex resulted in a c m r spectrum containing two sharp signals superimposed on the broad doublet Addition of sufficient tetraborate to form a 1 1 complex gave a spectrum having two sharp signals only This observation confirms that the tridentate (Fig 5, **10**, $R = Na^+$) form is produced exclusively, as any borate complex of type II or III formed from vicinal diols should give three signals or more

A tridentate complex was also formed on addition of boric acid to a solution of *cis*-inositol in D_2O An equivalent (1 1) amount of boric acid resulted in a c m r spectrum consisting mainly of a broad doublet, on which small sharp signals were superposed. The latter were enlarged on addition of boric acid in a 3 1 molar excess

1968	—														
1968	1938														
—	1938														
2223	—	—	—	2080	—	2040	—	—	—	1815	1798	1763	—	—	
2223	2185	2180	2128	—	2093	2043	1915	1895	1845	1815	1798	1765	1720	1685	
	2138	—	2005	1883		928	915								
	2138	2080	2008	1885		935	920								
	—	2078	—	1888		935	920								
—	—	2035	1980	—	930	918	818								
2060	2053	2035	1980	935	930	920	818								
2060	2053	—	—	938	—	923	818								

and had an area approximately one half that of the *cis*-inositol signals (Table III) The tridentate borate complex should have the novel structure **10**, $R = H^+$ (Fig 5)

epi-Inositol, in agreement with the findings of Angyal and McHugh[2,5], gave the 1 1 tridentate complex almost exclusively on addition of the cyclitol to sodium tetraborate in D_2O The c m r spectrum of the cyclitol displayed four signals, which were diminished by half in size on adjusting the inositol to available borate ratio to 2 1, and were replaced with 4 new signals (Table III) These signals became completely dominant when the ratio was adjusted to 1 1 C m r -spectral examination showed that *epi*-inositol did not form a complex with boric acid in D_2O

The c m r spectrum arising from *myo*-inositol in D_2O, which contains 4 signals, was modified by addition of sodium tetraborate and three new sharp signals were formed (Table III), at cyclitol to avilable borate ratios of 2 1 and 1·1 At the initial level of borate, the new signals had 10–20% of the magnitude of the signals of *myo*-inositol This observation contrasts with the c m r signals of the closely related

1,2,3/4-cyclohexanetetrol, which do not undergo changes in chemical shift on addition of borate Instead of forming type II or III complexes it therefore appears probable that the new signals from *myo*-inositol arose from a tridentate type IV complex

Formation of a tridentate complex was also demonstrated in the mannose series A di-*O*-methyl derivative of D-mannose was postulated by Urbas *et al* [15] to be the 3,5-di-*O*-methyl isomer because it migrated on paper electrophoresis in aqueous sodium tetraborate Since this compound was correctly identified later as 2,4-di-*O*-methyl-D-mannose[16], it appeared probable that a tridentate complex of structure **14** (Fig 5) was formed 2,4-Di-*O*-methyl-D-mannose in D_2O gave a c m r spectrum (Table III) showing a minor signal for C-1 of the β form (from **13**) at 2650 Hz, a major signal at 2568 Hz for C-1 of the α form (from **12**), and two OCH_3 signals at 1798 Hz and 1763 Hz After addition of sodium tetraborate, the spectra showed both the original signals and new ones from the complex Only one new compound was formed, as evidenced by the C-1 signal of the borate complex (**14**) at 2548 Hz and the OCH_3 signals, which were at 1720 Hz and 1685 Hz The diminution of the original signals on graded addition of sodium tetraborate to the D_2O solution could be monitored quantitatively by comparison with an internal standard In a solution containing a sugar to available borate ratio of 1 2, 2,4-di-*O*-methyl-α,β-D-mannose and **14** were present in a 2 3 ratio

Formation of a tridentate complex also appears possible with 1,2-*O*-isopropylidene-α-D-glucofuranose, as this compound can be readily converted into closely related 3,5,6-ortho esters of structure **11** (Fig 5) A tridentate structure was also suggested by Foster[8], following his observation that a complex having a high electrophoretic mobility is formed in aqueous sodium tetraborate In the present study, the c m r spectrum of the compound in D_2O was found to show 9 signals (Table III) At a ratio of sugar to available borate of 2 1 additional signals were formed and the former ones decreased by 50%, and when the ratio was adjusted to 1 1 a spectrum displaying 9 new signals was obtained, thus indicating the formation of a type II or IV complex The possibility of a type III complex being formed by consumption of only half the available borate was considered unlikely, as such a complex could exist in two isomeric forms and a larger number of signals would have been observed

The tendency to form a type II 3,5-complex in the related 6-*O*-isopropyl-1,2-*O*-isopropylidene-α-D-glucofuranose is marked By a series of experiments parallel to the foregoing ones, the 11 c m r signals of the isopropyl derivatives were replaced by 11 new signals when a level of sugar to available borate was 1 1 (Table III)

Observations on broadening of ^{13}C signals — As can be seen in the preceding discussion, two types of c m r spectral effects can occur on addition of borate to polyhydroxy compounds dissolved in D_2O One of these effects is that upon borate addition the chemical shifts of the signals of ^{13}C atoms remain unchanged, but broadening of signals of ^{13}C atoms in the vicinity of the borate complex occurs This effect is rather interesting and requires explanation

In general this spectral effect takes place with compounds that appear unlikely

to undergo conformational changes on conversion into their borate complexes These compounds include the 2-*O*- and 2,3-di-*O*-methyl derivatives of methyl α-D-mannopyranoside (Table I), and *cis*-cyclohexane-1,2-diol and other related compounds (Table III)

The broadening of certain signals to a line-width of 40 Hz suggests either the interconversion of two or more conformers or a chemical equilibration of compounds that is too rapid for each individual moiety to be detected by c m r spectroscopy In such cases an average type of spectrum should be obtained In the case of the mannosides forming complexes at O-4 and O-6, the former effect would occur if the borate ring underwent facile interconversion from one conformation to another Another, perhaps more attractive, possibility is that borate complexes of type II or type III interconverted so rapidly that they are not be individually detected by c m r spectroscopy, a process that could be facilitated by the favorable conformation of the sugar The free sugar is present in only relatively low concentration, as the line heights of the C-4 and C-5 signals (Fig 3) are very small compared with those in the c m r spectrum of the free sugar (The signals of *cis*-cyclohexane-1,2-diol and related compounds are not shortened to such an extent on addition of borate presumably because of the relatively low proportion of borate complex formed)

An observation that may possibly be related to this broadening phenomenon has been made by Garegg and Lindstrom[17] They recorded the p m r spectrum of the tridentate borate complex of *epi*-inositol and found that the signals of H-1, H-3, and H-5 (O-1, O-3, and O-5 are complexed by borate) appear as a broad unresolved signal of 0 3 p p m in width However this observation requires explanations different from those just given for the c m r spectral effect It has been shown in the present study, and also by Angyal and McHugh[2,5], that *epi*-inositol exists almost exclusively as its borate complex in D_2O or H_2O containing sodium tetraborate, and an average p m r spectrum would not be expected Also an average spectrum is inconsistent with the rigid conformation of the complex, and it is thus necessary to search for other explanations of the signal broadening

It is possible that quadrupole broadening of the signal of the nuclei such as ^{1}H and ^{13}C having spin ½ could occur if they are coupled to the ^{11}B nucleus of spin[18] 3/2 Work aimed at elucidating the phenomenon of ^{13}C signal broadening is being continued

EXPERIMENTAL

Nuclear magnetic resonance spectroscopy — C m r spectra were obtained by using a Varian XL-100-15 n m r spectrometer with Fourier transform on D_2O solutions (4 ml) in 12-mm tubes The sweep width was 5000 Hz, the acquisition time 0 4 sec, and the pulse width 50 μsec Chemical shifts are expressed in Hz and are based on the downfield difference of the resonance of the signal and that of the deuterium lock with a sweep offset of 32001 Hz The weights of carbohydrate and the number of transients (trans) used in each run, with and without borate or boric

acid, are given as follows The number of transients needed in the presence of borate were greater than in its absence because of diminution of resolution

Methyl 4,6-*O*-ethylidene-α-D-mannopyranoside[19] (200 mg, 30,000 trans), S–B* 2 1, 19,000 trans , S–B 1 1, 27,000 trans , S–B 1 2, 19,000 trans

Methyl 4-*O*-methyl-α-D-mannopyranoside[20] (37 mg, 84,000 trans.), S–B 2·1, 89,000 trans ; at S–B 1:1, 193,000 trans , at S–B 1 2, 360,000 trans.

Methyl 4,6-di-*O*-methyl-α-D-mannopyranoside[21] (70 mg, 8,000 trans)

Methyl 2-*O*-methyl-α-D-mannopyranoside[22] (86 mg, 27,000 trans), S–B 2 1, 137,000 trans ; S–B 1·2, 33,000 trans

Methyl α-D-mannopyranoside, supplied by Mann Research Laboratories Inc , (192 mg, 5,000 trans)

Methyl 2,3-di-*O*-methyl-α-D-mannopyranoside[23] (90 mg, 15,000 trans); S–B 1 2, 37,000 trans

Erythritan, obtained by deionization of its potassium borate complex[7] (80 mg, 15,000 trans), S–B 4 1, 20,000 trans , S–B 2 1, 11,000 trans , S–B 1 1, 38,000 trans , S–B 1 2, 40,000 trans

Methyl 5,6-di-*O*-methyl-α-D-mannofuranoside[16] (210 mg, 15,000 trans), S–B 2 1, 15,000 trans , S–B 1 1, 26,000 trans ; S–B 1·2, 28,000 trans

cis And *trans*-cyclohexane-1,2-diol, supplied by K and K Laboratories, Inc Plainview, N Y (80 mg, 30,000 trans), at S–B 2·1, 1 1 and 1·2, 30,000 trans

1,4,5,6-Tetra-*O*-methyl-*myo*-inositol[24] (80 mg, 25,000 trans), S–B 2 1, 31,000 trans ; S–B 1·2, 28,000 trans

1,2,3/4-(±)-Cyclohexanetetrol[25] (36 mg, 37,000 trans), at S–B 2 1, 42,000 trans , S–B 1 1, 52,000 trans

1,2 3,4-Di-*O*-isopropylidene-L-*chiro*-inositol[13] (180 mg; 4,000 trans , S–B 1 2, 6,000 trans

Ethylene glycol, supplied by Fischer Scientific Co (170 mg, 1,000 trans), S–B 1 1, 5,000 trans

1,2 5,6-di-*O*-Isopropylidene-D-mannitol[26] (260 mg, 10,000 trans), S–B 2 1 and 1 1, 20,000 trans , at boric acid to carbohydrate ratio of 3 1, 20,000 trans

cis-Inositol[27] (70 mg, 15,000 trans) S–B 2 1, 81,000 trans, S–B 1 1, 32,000 trans , S–B 1·2, 30,000 trans , at an inositol (36 mg) to boric acid ratio of 1 3, 122,000 trans

myo-Inositol, supplied by H. M Chemical Co. Ltd , Santa Monica, Calif (180 mg, 8,000 trans), S–B 2 1, 30,000 trans ; S–B 1·1, 27,000 trans ; S–B 1 2, 24,000 trans Similar values for *epi*-inositol[28]

2,4-Di-*O*-methyl-D-mannose[16] (30 mg, 52,000 trans), S–B, 2 1, 127,000 trans , S–B 1 1, 66,000 trans , S–B 1 2, 127,000 trans

1,2-*O*-Isopropylidene-α-D-glucofuranose[29] (220 mg, 19,000 trans), S–B, 2 1, 20,000 trans , S–B, 1.1, 40,000 trans , S–B 1 2, 50,000 trans

*S–B = ratio of substrate to available borate

6-*O*-Isopropyl-1,2-*O*-isopropylidene-α-D-glucofuranose[30] (184 mg, 15,000 trans); S–B 2 1, 15,000 trans ; S–B 1 1, 28,000 trans , S–B 1 2, 30,000 trans

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