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STRUCTURES OF THE BORATE COMPLEXES OF D-ALLOSE, D-TALOSE, AND D-PSICOSE IN AQUEOUS SOLUTION: AN ^{11}B- AND ^{13}C-N.M.R. STUDY

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ABSTRACT

The formation of 1:1 or 1:2 borate–sugar complexes by sugars having *ribo* configurations has been studied by ^{11}B- and ^{13}C-n.m.r. spectroscopy. Two 1:2 complexes can be formed, depending on whether the sugar is α or β. The main species involved *cis*-HO-1,2. D-Psicose formed a single complex at HO-2,3. A second species was formed by D-talose (10%), D-ribose (30%), and D-allose (30%), which involved *cis*-HO-2,3 with HO-1,2 *trans*. The order of stabilities of the complexes was D-psicose > D-ribose > D-talose > D-allose. The high affinity of *ribo* sugars towards borate is discussed. There was no correlation between the stability constants and the relative proportions of 1:2 complexes.

INTRODUCTION

In aqueous solution, most sugars react with borate ions (B^-) forming 1:1 (BL^-) and 1:2 (BL_2^-) complexes[1] according to the reactions

$$B^- + L \rightleftarrows BL^- \qquad \beta_1 = (BL^-)/(B^-)(L)$$
$$B^- + 2\,L \rightleftarrows BL_2^- \qquad \beta_2 = (BL_2^-)/(B^-)(L)^2$$

in which BL_n^- stands for the 1:n complex formed with a ligand L.

Such complexes are useful in chromatographic separations[2–4] of sugars, using anion-exchange columns. The order of elution can be predicted[5] when the stability constants β_n are known. We have interpreted[6] the structures and stabilities of the borate complexes of the four aldopentoses by showing that the sugars were com-

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plexed in the furanose form. The discussion was extended to the aldohexoses and ketohexoses by using the concept of isoconfigurational series, *i.e.*, sugars with identical structures in the neighbourhood of the anomeric carbon. However, in most series, only one aldohexose could be studied, since the other one was not available commercially.

It has been reported[7] that borate ions partially protected D-galactose against ionizing radiations and, since the borate complexes of D-ribose are much more stable[6] than those of D-galactose, and D-ribose has a central role in biology, we have investigated the reaction of borate with sugars of the *ribo* series, namely, D-ribose, D-allose, D-talose, and D-psicose.

RESULTS

^{11}B-N.m.r. spectroscopy is now a well-established tool[6,8–10] for studying the structures of borate–sugar complexes. Boron exchange between complexed and uncomplexed boric species is slow on the n.m.r. time-scale and separate signals are obtained for the different species in equilibrium. In addition to the signal of boric acid (δ 0, reference), the ^{11}B-n.m.r. spectra of the borax–sugar mixtures contained two new signals, assigned to the 1:1 and 1:2 complexes according to literature data (Table I). As expected, the signals for the 1:2 complexes were broader than those of the 1:1 complexes, because the former complexes were larger. The δ values obtained were in the range generally observed[6,8–10], which is characteristic of complexes involving *vicinal* diol groups *i.e.*, involving a five-membered ring either in 1:1 or 1:2 species. The formation of six-membered rings would have given a signal[9] at δ ~18.

Aqueous solutions of the above sugars contained a mixture of α and β pyranose and furanose forms, except for D-allose, the furanose form of which was not observed, as shown by comparison of their ^{13}C-n.m.r. spectra with literature data[11–15]. After the addition of borax (sugar–borate ratio, 2; acidic medium), ^{11}B-n.m.r. spectroscopy showed that 1:2 complexes were the preponderant species and

TABLE I

^{11}B-N.M.R. CHEMICAL SHIFTS AND LINE-WIDTHS AT ~26° FOR BORATE COMPLEXES[a] OF ALDOSES AND KETOSE OF THE *ribo* SERIES

Sugar	δ (*p.p.m.*)[b]		$\Delta\nu$ (*Hz*)	
	BL^-	BL_2^-	BL^-	BL_2^-
D-Ribose	−12.9	−7.9	36	57
D-Allose	−12.5	−7.8	42	~100
D-Talose	−12.4	−7.5	36	~100
D-Psicose	−12.6	−8.2	36	52

[a]0.5M Borate, M sugar. [b]Referenced to external boric acid. Accuracy: δ ±0.1 p.p.m.; $\Delta\nu$ ±5 Hz.

^{13}C-n.m.r. spectroscopy was used to determine the sites of chelation. The carbons bearing the hydroxyl groups involved in chelation are deshielded[6,8–10,16] and we have found similar results. D-Psicose formed a single complex quantitatively, since the signals for the free ketose were almost absent and six ^{13}C signals for the complex could be observed. For the aldoses, uncomplexed sugar was detected (Table II) and there were two other species, each of which had six ^{13}C resonances. The corresponding complexes were designated as follows. The subscript 1 indicates the major complex present in the proportion given in brackets: D-ribose, R_1 (70%) and R_2 (30%); D-allose, A_1 (70%) and A_2 (30%); D-talose, T_1 (90%) and T_2 (10%).

It was concluded that D-ribose, D-allose, and D-talose each formed two different types of 1:2 borate complex, but the structures were similar since there was only one ^{11}B signal for these complexes.

Table III presents the ^{13}C chemical shift data for the 1:2 borate–sugar complexes and the assignments. Data for D-ribose are given for comparison, since the variations of the n.m.r. parameters of the ligand carbons upon complexation accord with furanose forms only. Clearly, the single psicose complex was related to the ribose R_1 complex and was designated by P_1. The marked analogies for the type 1 complexes (R_1, A_1, T_1, P_1) indicate similar structures. Likewise, the type 2 complexes (R_2, A_2, T_2) had similar structures. Thus, it was inferred that, like D-ribose, all hexoses of the series were complexed in the furanose form.

The variations ($\Delta\delta$) of chemical shifts due to complexation were calculated for the different sugars (Table III). Besides the expected shielding of the carbons bearing the chelating hydroxyl groups, complexation of sugars of the *ribo* series also resulted in high $\Delta\delta$ values for the resonance of C-4 (C-5 in D-psicose), which bears the side chain. The resonance of this carbon was shifted by 4.5 p.p.m. in type 1 complexes, and by ~5 p.p.m. in type 2 complexes. Since C-4 does not carry a

TABLE II

RELATIVE STABILITIES OF 1:2 BORATE–SUGAR COMPLEXES BASED ON ^{13}C-N.M.R. DATA

Aldose	*Complexed*[a,b] *(%)*	*Free (%)*	*log* β_2
D-Arabinose	30	70	2.99[c]
D-Lyxose	50	50	3.39[c]
D-Xylose	80	20	3.74[c]
D-Ribose	95	5	4.80[c]
D-Galactose	20	80	2.56[c]
D-Mannose	20	80	2.74[c]
D-Glucose	55	45	3.05[c]
L-Sorbose	95	5	5.75[c]
D-Allose	85	15	3.9 to 4.4[d]
D-Talose	90	10	4.2 to 4.7[d]
D-Psicose	>99	<1	≥6[d]

[a]The percentages were calculated (±5%) from the intensities of the ^{13}C signals: M sugar, 0.5M sodium borate. [b]Mainly 1:2 complexes: <10% of 1:1 complex (^{11}B-n.m.r. data). [c]Determined by potentiometry[6]. [d]Estimated in this work (see text).

TABLE III

^{13}C-N.M.R. CHEMICAL SHIFTS (IN P.P.M.) OF SUGARS OF THE *ribo* SERIES AND THEIR BL_2^- BORATE COMPLEXES

Sugar	*Carbon*[a]					
D-Ribose[b] (L)	1	2	3	4		5
δ in complex R_1	103.0	77.2	72.2	79.9		61.7
δ in L(α-*f*)	97.4	72.2	71.1	84.2		62.5
$\Delta\delta$	5.6	5.0	1.1	−4.3		−0.8
δ in complex R_2	104.3	83.8	78.6	88.3		64.2
δ in L(β-*f*)	102.1	76.4	71.7	83.6		63.7
$\Delta\delta$	2.2	7.4	6.9	4.7		0.5
D-Talose (L)	1	2	3	4	5	6
δ in complex T_1	102.8	77.0	72.6	79.4	73.6	64.4
δ in L(β-*f*)	98.0	72.3	72.1	83.9	72.6	64.4
$\Delta\delta$	4.8	4.7	0.5	−4.5	1.0	0.0
δ in complex T_2	104.8	83.9	80.3	89.0	74.7	63.9
δ in L(α-*f*)	102.4	76.7	72.1	83.3	73.3	64.3
$\Delta\delta$	2.4	7.2	8.2	5.7	1.4	−0.4
D-Allose (L)	1	2	3	4	5	6
δ in complex A_1	102.9	77.2	72.9	79.7	73.8	63.8
δ in L(α-*f*)	96.8	72.4	72.5	84.3	70.2	63.1
$\Delta\delta$	6.1	4.8	0.4	−4.6	3.6	0.7
δ in complex A_2	104.2	83.7	78.7	88.5	73.8	64.1
δ in L(β-*f*)	101.6	76.1	71.7[c]	83.0	73.3[c]	63.3
$\Delta\delta$	2.6	7.6	7.0	5.5	0.5	0.8
D-Psicose (L)	1	2	3	4	5	6
δ in complex P_1	65.1	110.8	78.2	72.3	80.8	61.8
δ in L(α-*f*)	65.5	104.6	71.5	71.7	84.0	62.6
$\Delta\delta$	−0.4	6.2	6.7	0.6	−3.2	−0.8

[a]Carbons appearing in the same column are structurally equivalent. Assignments from the literature for D-ribose[11], D-talose[12,13], and D-psicose[14]. Chemical shifts for uncomplexed D-allofuranoses were taken from ref. 15. [b]Results and assignments for D-ribose complexes were taken from ref. 6. [c]These assignments were reversed on the basis of structural similarities.

hydroxyl group in the furanose form, such effects must be ascribed to small changes in the sugar ring conformation due to complexation.

Type 1 complexes are formed when HO-1,2,3 are *cis*. For this structure, complexation involves HO-1,2 only, probably due to the higher reactivity of the anomeric hydroxyl group. Type 2 complexes are formed when HO-1,2 are *trans*. The only available *cis*-diol group is HO-2,3, and its complexes are formed in smaller proportion and so appear to be less stable than the former in the *ribo* series.

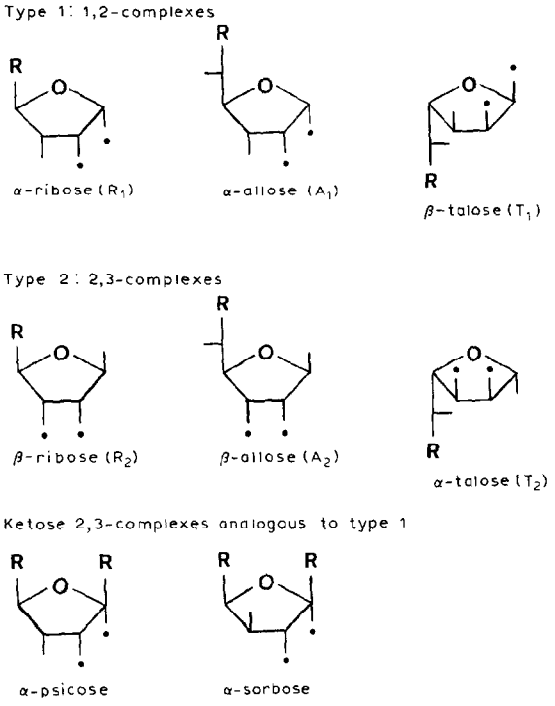

Fig. 1. Structures of the ligands in 1:2 borate–D-carbohydrate complexes: R = CH_2OH and ● indicates the site of chelation.

Unlike D-ribose and D-allose, which formed similar ratios of type 1 (70%) and type 2 (30%) complexes, D-psicose did not form a type 2 complex. It is possible that, in the hypothetical P_2 complex (involving β-D-psicofuranose), the borate moiety chelated by OH-3,4 would be *cis* to the CH_2OH-1, with consequent steric repulsion. However, this argument is not valid for D-talose, which formed up to 90% of the T_1 species. The structures of the complexes R_1, A_1, T_1, and P_1 (Fig. 1) show that the borate moieties are always oriented *trans* to the group attached to C-4 (C-5 for psicose). The only apparent reason for the peculiar behaviour of D-talose was found by considering the C-4 side-chain, in which HO-5 has different orientations in allose and talose. An analogous contribution of HO-6 to the stability of complexes has been found by comparing[6] mannose and rhamnose.

Although α and β anomers can interconvert easily, complexation did not shift the equilibrium to a single form of D-talose, indicating that the T_1 and T_2 complexes have similar stabilities. In contrast, D-psicose was completely converted into the α-furanose form on complexation with borate.

The small amounts (250 mg) of the sugars available did not allow accurate determination of the stability constants (log β_n) for their complexes, either by potentiometry or n.m.r. spectroscopy. The β_1 constants depend little on sugar con-

figurations[6]. For all the aldoses studied hitherto, log β_1 = 2.05 ±0.25, and no special behaviour was expected in the *ribo* series. In contrast, β_2 values can vary over a considerable range and an attempt was made to define at least the order of magnitude of complex stabilities by comparing the proportions of uncomplexed ligands in borax solutions under identical conditions. The data in Table II show a qualitative correlation between this proportion and the stability constant (log β_2) for a selection of aldopentoses and aldohexoses. The estimated stability constants of the 1:2 borate complexes of D-allose and D-talose were obtained by interpolation. For D-psicose, the data had to be extrapolated, since the 1:2 complex was more stable than those of any sugar studied earlier, so that a lower limit ($\geq$6) is proposed for the stability constant. The strongest complex known[6] before was that of L-sorbose (log β_2 5.75).

An unexpected result was that D-allose and D-talose formed 1:2 borate complexes with stabilities much higher than those of other aldohexoses and even of D-xylose. The earlier belief[6] that aldohexoses formed complexes of low stabilities appears then to reflect insufficient experimental data.

DISCUSSION

The origin of the high affinity of sugars in the *ribo* series for borate is of interest. The relationship between the stabilities (as log β_2) of the 1:2 complexes and the structures of the sugars can be rationalized[6] by considering the nature and orientation of the substituents on the furanose ring. The formation of a cyclic borate at HO-1,2 of the furanose form (the favoured site) can be reduced (*a*) by interaction with the side chain if it is *cis* and (*b*) if HO-3 and the side chain are *cis* (this is the main[17] unfavourable interaction in furanoses). Only in sugars of the *ribo* series are both of these effects absent. In the *xylo* series, the site of complexation is also *trans* to the side chain, but there is little furanose in the equilibrium owing to the HO-3–side-chain interaction.

Type 2 complexes cannot form in the *xylo* series, because HO-2,3 are *trans*. The possibility of forming both type 1 and type 2 complexes might make the complexes in the *ribo* series more stable. It has been suggested[18,19] that borate complexes of higher stabilities are formed when several chelating diol groups are available in the ligand. For example, D-ribose was assumed[18] to form simultaneously 1,2-, 2,3-, 3,4-, 1,3-, and 2,4-pyranose complexes. ^{11}B-N.m.r. studies[9,20] substantiated this idea by demonstrating that borate formed complexes with β-diols such as 1,3-propanediol. Our results[6] did not support this hypothesis, because sugars were shown to complex in the furanose form and not in the pyranose form, and two 1:2 complexes at most were detected for the sugars studied. The present results are more conclusive since no correlation was observed between the relative proportions of type 1 and type 2 complexes and the overall stability constants log β_2. D-Psicose formed a single 1:2 species (the bis-2,3-α-D-psicofuranose complex) but, nevertheless, displayed the highest affinity for borate.

Thus, the order of reactivity *ribo* ⩾ *xylo* was attributed to the difference in the configuration at C-3 (C-4 in ketoses). This effect was studied by comparing D-psicose (*ribo* series) and L-sorbose (*xylo* series), each of which forms only one type 1 complex, in order to avoid misinterpretations due to the presence of type 2 complexes. The psicose complex, in which HO-4 is *trans* to both CH_2OH groups and *cis* to the borate moiety, is more stable than the sorbose complex, in which the configuration at C-4 is inverted. This finding suggests that HO-4 in psicose stabilises the borate complex. The expected steric interaction of borate and HO-4 appeared to have a negligible effect on the stability of the 1:2 psicose complex.

Consideration of the conformations rationalizes the present and earlier[6] results, since there seems to be a direct relationship between the ratios of *cis* and *trans* furanose anomers in the uncomplexed sugars and the ratios of type 1 *(cis)* and type 2 *(trans)* in their 1:2 borate complexes. For ribose, the *cis:trans* ratio is 1:2 because of the interaction between HO-1 and HO-2 in the former[21]. Chelation by borate reduces this interaction and changes the ratio to 7:3, corresponding to a gain in free energy of ~4 $kJ.mol^{-1}$ for the *cis* ligand. The behaviour of allose is similar. In contrast, for psicose, the *cis* anomer is already preponderant[14] (38:15) and a similar energy gain will bring it to >90%. In talose, the *trans* form is disfavoured[22] (13:16) by the interaction of HO-1 and HO-5. This interaction is accentuated on flattening the ring at C-2,3; hence, in the borate complex, the ratio becomes 9:1. Similarly, the strong affinity of ketoses for borate may be due to the *cis* form of the furanose preponderating over the *trans* form before the formation of complexes.

Thus, it is clear that the sugars of the *ribo* series form 1:2 borate complexes that are more stable than those of related sugars of the other series. The conclusions[6] for the aldopentoses can be applied to the whole series, for which the relative order of affinity towards borate is *ribo* ⩾ *xylo* > *lyxo* > *arabino*. Within all series, the order of stabilities is ketohexose > aldopentose > aldohexoses. More generally, the concept of isoconfigurational series is confirmed as a valuable guide in studies of carbohydrate complexes. A consequence is that D-galactose, which belongs to the *arabino* series, was probably not a suitable model[7] for studying the protective effect of borate against radiations. More important variations should be observed with members of the *ribo* series. It should be noted that adenosine forms[16] a borate complex, the structure of which appears to be similar to that of the β-D-ribofuranose R_2 species.

EXPERIMENTAL

The sugars studied were commercial products. ^{11}B-N.m.r. spectra were recorded at 28.88 MHz with a Bruker WH 90 C spectrometer, and ^{13}C-n.m.r. spectra at 20.11 MHz with a Bruker WP 80 spectrometer. All solutions (1 mL) in D_2O were M with respect to sugar and 0.5M with respect to sodium borate. The experimental techniques have been described[6].

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