Tetrahedron Vol. 44, No. 14, pp. 4469 to 4482, 1988
Printed in Great Britain.

0040-4020/88 $3.00 + .00

A ^{11}B AND ^{13}C NMR DETERMINATION OF THE STRUCTURES OF BORATE COMPLEXES OF PENTOSES AND RELATED SUGARS

Stella CHAPELLE[*,a] and Jean-François VERCHERE[b]

[a]Laboratoire de RMN de l'Université de Rouen,

[b]Laboratoire de Chimie Macromoléculaire, U.A. 500 du CNRS,

Faculté des Sciences, B.P. 118, 76134 MONT-SAINT-AIGNAN CEDEX, FRANCE.

(*Received in Belgium* 22 *April* 1988)

Abstract: A ^{11}B NMR study demonstrated that the four pentoses, like hexoses, form concurrently 1:1 and 1:2 borate complexes in aqueous solution, contrary to earlier reports. It also showed that in both species, the borate ion was bound to a vicinal diol site. The corresponding stability constants were determined by a potentiometric method. The structures of most 1:2 complexes were precised by ^{13}C NMR spectroscopy, since the carbon atoms which bear the chelating diol group were clearly deshielded and had increased $^{1}J_{CH}$ values. The results were rationalised by grouping the sugars in series of related configurations. All sugars were complexed in furanose form. Those of the arabino-galacto-fructo series and of the xylo-gluco-sorbo series gave the same type of complex involving the anomeric hydroxyl group and the nearest ring CHOH. The very stable 1:2 complex of D-ribose was shown to be a mixture of two species in constant ratio, borate being bound either at C-1,C-2 or at C-2,C-3. The same result was obtained with the sugars of the lyxo-manno-tagato series, which also possessed two cis CHOH groups.

In aqueous solution, borate ions react with polyhydroxy compounds to form ionized complexes which have received several applications in carbohydrate chemistry, for example in the chromatographic determination of mixtures of sugars using anion-exchange resins[1,2] and for possible protection[3] against ionizing radiations. Moreover, borate ions are known to catalyze[4] the isomerization of aldoses to ketoses in alkaline media.

The very stable borate complexes of ketoses and alditols (especially D-mannitol[5]) have been the subject[6,7] of numerous studies. The classical scheme of complex formation is :

$$B^- + L \rightleftarrows BL^- \qquad \beta_1 = (BL^-)/(B^-)(L)$$

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

in which B^- stands for the borate ion and BL_n^- for the 1:n complexes formed with a ligand L.

In contrast, much less is known about the weaker aldose complexes. Several papers report conflicting results[6-11] on the stability constants β_n and even on the compositions of

pentose complexes. In some cases, the selective formation of 1:1 or 1:2 species was claimed[10] to depend on the configuration of the ligand. We therefore undertook a comprehensive study of the stability of borate complexes of simple sugars, using the improved potentiometric method[11] recently developed for the study of oligosaccharide complexes.

The structures of borate complexes have been discussed from comparisons of thermodynamical data[10-12] within series of ligands possessing related configurations. Recently, a combination of ^{11}B and ^{13}C NMR spectroscopies has proved to be a powerful tool for the elucidation of the nature of complex formation between borate and polyhydroxy ligands[13-15] in aqueous solution. In the present study, these methods were applied to the pentose series in order to relate the stabilities of the complexes to the structures of the sugars. When available, related hexoses (aldoses or ketoses) were studied for comparison. Our main goals were the determination of the form, pyranose or furanose, in which the sugar was complexed and the identification of the chelating hydroxyl groups.

EXPERIMENTAL

All the sugars were commercial compounds (Fluka or Aldrich) of the highest available grade, used without further purification. The preparation of borax solutions and the determination of stability constants have been previously described.[11]

^{11}B NMR.- The spectra were recorded at 28.88 MHz using a BRUKER WH 90 C multinuclei spectrometer. The temperature was about 26°C. The sample concentrations were 1 $mol.l^{-1}$ (borax) and 0.5 $mol.l^{-1}$ (sugar) in D_2O (1 cm^3). The chemical shifts have been measured by the substitution method[16], with boric acid as reference.

^{13}C NMR.- The measurements were made at 20.11 MHz using a BRUKER WP 80 spectrometer. The samples were those prepared for the ^{11}B NMR study and were used within a week (the spectra did not vary during this interval). The temperature was about 28°C. The spectra were referenced externally to the C_6D_6 signal at 128 ppm.

For decoupled spectra, a pulse angle of 90° and a relaxation delay of 3 s were used. The proton-coupled spectra were obtained with nuclear Overhauser enhancement by gating off the decoupler during data acquisition. The resolution was improved by applying an apodisation function to the free-induction signal before Fourier transformation.

RESULTS AND DISCUSSION

^{11}B NMR. The principle[13] lies on the facts that hydroxyl exchange between boric acid and borate is fast on the ^{11}B time scale and gives only one mean signal for uncomplexed boron. On the contrary, boron exchange between boric acid and its complexes is slow on the ^{11}B time scale and a separate signal can be observed for each species.

Table 1 displays ^{11}B chemical shift data for borate complexes of a series of ketoses and aldoses. The signals corresponding to the 1:1 complexes ($\delta \approx -13$ ppm, sharp) and the 1:2 complexes ($\delta \approx -8.5$ ppm, broad) could be assigned in agreement with previous studies[13-15]. The values of the ^{11}B chemical shifts in both series of complexes, which varied little with the nature of the sugar, allowed us to conclude that the chelating site was always a vicinal diol group, forming a five-membered ring with the boron atom. Complexes of β-diol groups would have given[13] signals at $\delta < -18$ ppm.

The large differences in the linewidths (Table 1) of the two series of complexes can be attributed to their different molecular radii. Accordingly, the signals given by the disaccharide lactulose were found to be particularly broad.

Stability constants. Despite several potentiometric studies[9-10] which claimed that pentoses form only one borate complex, with 1:1 or 1:2 stoichiometries depending on the sugar

TABLE 1 - ^{11}B chemical shifts and linewidths for borate complexes of sugars

ligand	δ (ppm)[a] BL^-	δ (ppm)[a] BL_2^-	Δν (Hz) BL^-	Δν (Hz) BL_2^-
D-lyxose	-12.7	-8.1	32	48
D-xylose	-12.8	-8.3	70	≈ 140
D-arabinose	-13.1	-9.1	24	80
D-ribose	-12.9	-7.9	36	57
D-galactose	-13.0	-8.8	15	≈ 110
D-glucose (*)	-12.9	-8.4	64	≈ 150
D-mannose	-12.4	-8.0	48	60
D-tagatose	-13.0	-8.8	ND	≈ 150
L-sorbose	-12.5	-8.5	45	81
D-fructose (*)	-12.9	-8.9	ND	70
lactulose	-12.3	-8.3	≈ 100	≈ 140

[a] chemical shifts with boric acid as external reference. ND : not determined.
t ≈ 26°C ; concentrations : borax 0.5 $mol.l^{-1}$; ligand 1 $mol.l^{-1}$
(*) literature[15] values for BL_2^- : -8.7 ppm (D-glucose), -9.5 ppm (D-fructose).

TABLE 2 - Overall stability constants[a] of borate complexes of sugars

ligand	This work log β_1	This work log β_2	literature (ref) log β_1	literature (ref) log β_2	
D-ribose	2.26	4.80	ND	7.20	(9)
			ND	3.01	(17)
D-xylose	1.95	3.74	ND	4.01	(8)
D-lyxose	2.15	3.39	4.82	ND	(9)
D-arabinose	2.14	2.99	ND	3.28	(8)
			2.19	3.02	(7)
L-arabinose	2.10	2.97	ND	3.55	(8)
			2.11	2.83	(6)
L-rhamnose [b]	1.58	2.14	ND	2.61	(8)
deoxy-2-ribose [c]	0.92	1.60	3.85	ND	(10)
D-glucose	1.80	3.05 [d]			
D-galactose	1.99	2.56 [d]			
D-mannose	2.01	2.74 [d]			
D-fructose	2.82	4.97 [d]			
L-sorbose	< 3.5	5.75	ND	5.80	(8)

[a] determined by potentiometry[11] ; accuracy : log β_1 ± 0.08 ; log β_2 ± 0.08
t = 25°C ; I = 0.1 $mol.l^{-1}$ (KCl) ; ND : not determined.
b : 6-deoxy-L-mannose ; c : 2-deoxy-D-arythro-pentose
d : taken from ref.11, in which literature results were discussed.

configuration, the ^{11}B NMR results presented above show unambiguously that both 1:1 and 1:2 species are formed concurrently in every borate-sugar system, including those of pentoses. Accordingly, other potentiometric procedures[6,7,11] were shown to allow the determination of both stability constants.

An exception was found in this work for L-sorbose. Although this ketose formed two complexes detected in the ^{11}B NMR study (Table 1) the stability constant β_1 of the BL^- complex could not be determined by potentiometry, probably because, at low concentrations of ketose, the reaction with borate was slow and precluded the obtention of accurate pH values. Nevertheless, we performed simulated computations which showed that the log β_1 value could not be > 3.5, since such a stable 1:1 complex would have been detected. The failure of the method to detect the 1:1 species is therefore a consequence of the large stability difference between the 1:1 and the 1:2 species.

For all other compounds investigated, values of the stability constants β_1 and β_2 of the 1:1 and 1:2 complexes were determined (Table 2) by our original potentiometric procedure, the characteristics of which have been discussed[11] elsewhere. As before[11] the slow equilibration of borate-sugar mixtures was attributed to sugar reactions like mutarotation or pyranose-furanose interconversions. Some results are now commented.

- Both ketoses, D-fructose and L-sorbose, formed 1:2 complexes of higher stability than any aldose. Pentose complexes were also found more stable than those of C_6 aldoses. It gives the stability sequence of borate complexes : C_6 ketoses > C_5 aldoses > C_6 aldoses.

- We were unable to find any significant difference between the stability constants of the complexes of both arabinose enantiomers, which seems more logical than the contrary result of another paper[8]. Arabinose complexes were studied earlier and our results agree nicely with those of two other studies[6,7] which used a similar potentiometric method.

- Our values for pentose complexes are very different from those of other workers[8-10] who used a differential potentiometric method based on measurements of the buffer capacity of the solutions. In such studies, the oversimplifying assumption that only one complex was formed may have biassed the calculations of the stability constants.

- The remarkable stability of the 1:2 complex of D-ribose compared to other pentoses[10] is confirmed. However, earlier papers[10,17] reported very different values for its stability constant. A possible reason for the discrepancy could be that D-ribose formed a mixture of two 1:2 complexes, as is shown below. Besides, we found that the values calculated from several experiments were perfectly reproducible.

- The involvement of the C-2 hydroxyl of D-ribose in complex formation is attested by (i) the much lower stabilities of the homologous complexes of deoxy-2-D-ribose and (ii) the lower stability of the 1:2 D-arabinose complex, in which the C-2 configuration is reversed.

^{13}C NMR. Voelter and coll.[16] seem to have been the first to use ^{13}C NMR in the study of borate complexes of carbohydrate derivatives. These authors determined the variations of the chemical shifts for each carbon of the ligand and observed that those bearing the complexing hydroxyl groups were the most deshielded. A similar procedure was applied to the borate complexes of polyols[15] and fructose[15,19].

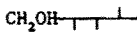
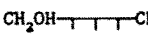
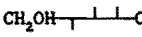
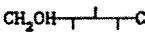
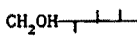
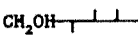
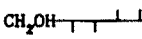
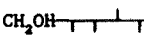
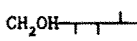
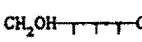
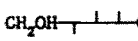
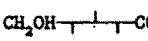
Since in our experimental conditions, the proportions of the 1:1 complexes were generally low, the investigations were limited to solutions prepared by mixing the sugars and borax in ligand/borate ratio = 2. The solutions were thus acidic and, because of their higher stabilities, BL_2^- complexes were the prevailing species.

In addition, we measured the direct coupling constants $^1J_{CH}$ for all carbons, except when the spectra were too complicated. As expected, they were found to increase for the carbons bearing the chelating hydroxyls, and remained almost unchanged for other carbons.

The study was rationalized by classifying the sugars into series (Table 3) of analogous configurations at C-1 to C-4 (aldoses) or C-2 to C-5 (ketoses). In each series, the ^{13}C signals of these carbons had similar values[20-24], in pyranose forms as well as in furanose

forms. Moreover, we observed that the spectra of the BL_2^- complexes in a given series were also very similar. Consequently, the variations of chemical shifts should be the same within a series, suggesting that the corresponding complexes had identical structures.

TABLE 3 - List of sugars forming series of analogous configurations.

arabino	ribo	lyxo	xylo
D-arabinose	D-ribose	D-lyxose	D-xylose
			
D-galactose	D-talose(*)	D-mannose	D-glucose
			
D-fructose	D-psicose(*)	D-tagatose	D-sorbose
			

(*) sugars not investigated

a) complexes of D-fructose and derivatives

Two ^{13}C NMR studies[15,19] of the BL_2^- complex of D-fructose established the existence of a bis (2,3-β-furanose) complex, although the uncomplexed sugar existed mainly in the β-pyranose form. Additional NMR evidence was provided by a comparison[15] with a disaccharide (isomaltulose) in which the fructose moiety was blocked in the β-furanose form, and one of us showed by a potentiometric study[11] that fructose-containing disaccharides needed free hydroxyls at C-2 and C-3 to give borate complexes as stable as those of D-fructose.

We began this work by comparing the BL_2^- complexes of D-fructose and lactulose (4-O-β-D-galactopyranosyl-β-D-fructofuranose). The results (Table 4) gave conclusive evidence that in lactulose, the borate anion was not bound to the galactose ring, but to the C-2 and C-3 hydroxyls of the β-fructofuranose ring. The corresponding variations of chemical shifts were found very close to those observed for D-fructose itself.

b) complexes of D-arabinose and related sugars

As indicated above, D-arabinose and D-galactose have configurations related to that of D-fructose. These aldoses exist in solution as the four possible forms : α and β pyranoses (>97%) and furanoses (<3%). After borax addition, the spectra indicated the presence of only one 1:2 complex for both sugars. The complexes could not involve the ligands in either pyranose form, since too many carbons would be strongly deshielded (especially C-4) contrary to the fact that borate is chelated by two hydroxyls only. An example of calculation is given (Table 5) for D-arabinose. The analogy with the D-fructose complex spectrum (Table 4) suggested instead that all three sugars were complexed in furanose form. One of these forms (α-D-arabinofuranose and β-D-galactofuranose) could be eliminated because the calculated deshielding effects (Table 5) would have been very small. Besides, assuming the ligands to be β-D-arabinofuranose and α-D-galactofuranose, calculations of the variations of chemical shifts gave values close to those obtained for D-fructose and lactulose. The most deshielded

TABLE 4 - ^{13}C chemical shifts in BL_2^- borate complexes of lactulose (4-O-β-D-galactopyranosyl-β-D-fructofuranose) and β-D-fructofuranose.

moiety = L	carbon position					
β-D-galactopyranosyl	1	2	3	4	5	6
δ (ppm) in L	101.9	71.8	73.6	69.7	76.4	62.2
δ (ppm) in BL_2^-	101.9	71.9	73.4	70.0	76.4	62.2
Δδ (ppm)	0	0.1	-0.2	0.3	0	0
β-D-fructofuranosyl	1	2	3	4	5	6
δ (ppm) in L	63.7	103.8	75.9	85.1	81.1	64.0
δ (ppm) in BL_2^-	65.1	112.2	83.2	86.7	85.0	63.2
Δδ (ppm)	1.4	8.4	7.3	1.6	3.9	-0.8
β-D-fructofuranose	1	2	3	4	5	6
δ (ppm) in L	63.9	102.5	76.6	75.7	81.7	63.3
δ (ppm) in BL_2^-	64.4	112.1	84.9	78.3	86.0	62.3
Δδ (ppm)	0.5	9.6	8.3	2.6	4.3	-1.0
Δδ (ppm) litt[15]	1.5	9.2	8.0	2.7	4.6	-0.3
Δδ (ppm) litt[18]	-0.5	9.2	8.2	2.8	5.0	ND

Assignments for ligands from ref 20-24. ND : not determined.

TABLE 5 - ^{13}C chemical shifts and coupling constants $^1J_{CH}$ of aldoses having the arabino configuration and of their BL_2^- borate complexes.

ligand = L	carbon position					
D-arabinose	1	2	3	4		5
δ (ppm) in BL_2^-	104.3	83.9	78.0	86.7		63.2
δ (ppm) in L(β-fu)	95.2	76.8	75.7	82.8		63.5
Δδ (ppm)	9.1	7.1	2.3	3.9		-0.3
δ (ppm) in L(α-fu)	102.1	82.1	76.8	82.8		62.8
Δδ (ppm)	2.2	1.8	1.1	3.9		0.4
δ (ppm) in L(α-py)	97.8	73.1	73.7	69.6		67.4
Δδ (ppm)	6.5	10.8	4.3	17.1		-4.2
δ (ppm) in L(β-py)	93.7	69.7	69.7	69.7		63.5
Δδ (ppm)	10.6	14.2	8.3	17.0		-0.3
D-galactose	1	2	3	4	5	6
δ (ppm) in BL_2^-	103.8	84.0	77.9	85.5	71.9	63.9
δ (ppm) in L(α-fu)	96.0	77.4	75.3	82.3	72.7[a]	63.5
Δδ (ppm)	7.8	6.6	2.6	3.2	-0.8	0.4
δ (ppm) in L(β-fu)	102.1	81.9	76.6	83.1	72.7	63.8
Δδ (ppm)	1.7	2.1	1.3	2.4	-0.8	0.1

Aldose assignments from ref 20-24. [a] uncertain assignment.

carbons were those of the cis-diol system involving the anomeric hydroxyl at C-1 and its neighbour at C-2. It is of interest to note that β-D-arabinose was also reported[25] to react in furanose form with B_2O_3 in $(CD_3)_2SO$.

c) complexes of D-ribose

Unlike other pentoses, D-ribose could not be compared to structurally related sugars as they are not commercially available. The ^{13}C NMR spectrum of D-ribose indicates that all four forms (α and β pyranoses and furanoses) are present at equilibrium. After the addition of borax, all the signals of free D-ribose almost disappeared and 10 new resonances appeared,

TABLE 6 - ^{13}C chemical shifts and coupling constants $^{1}J_{CH}$ of D-ribose and of its two BL_2^- borate complexes.

ligand	carbon position				
D-ribose = L	1	2	3	4	5
δ (ppm) in complex R_1	103.0	77.2	72.2	79.9	61.7
$^{1}J_{CH}$ (Hz) in complex R_1	180	154	146	148	142
δ (ppm) in L(α-fu)	97.4	72.2	71.1	84.2	62.5
$\Delta\delta$ (ppm)	5.6	5.0	1.1	-4.3	-0.8
$^{1}J_{CH}$ (Hz) in L(α-fu)	169.5	≈ 142	≈ 142	≈ 142	141
ΔJ (Hz)	10.5	12	4	6	1
δ (ppm) in complex R_2	104.3	83.8	78.6	88.3	64.2
$^{1}J_{CH}$ (Hz) in complex R_2	170	156	154	142	140
δ (ppm) in L(β-fu)	102.1	76.4	71.7	83.6	63.7
$\Delta\delta$ (ppm)	2.2	7.4	6.9	4.7	0.5
$^{1}J_{CH}$ (Hz) in L(β-fu)	169.5	142.5	145.5	142.5	141
ΔJ (Hz)	0.5	13.5	8.5	-0.5	-1
δ (ppm) in L(α-py)	94.6	71.1	70.3	68.4	64.2
$^{1}J_{CH}$ (Hz) in L(α-py)	≈ 169	142	142	144	142.5
δ (ppm) in L(β-py)	94.9	72.2	70.3	68.4	64.2
$^{1}J_{CH}$ (Hz) in L(β-py)	160	142.5	142	144	141

Assignments for D-ribose from ref 20-24.

which could be attributed (Table 6) to two different 1:2 borate complexes formed in unequal ratio, denoted hereafter R_1 (≈ 70%) and R_2 (≈ 30%) on the basis of signal intensities. It should nevertheless be recalled that only one signal (δ = -7.9 ppm) in the ^{11}B spectrum could be attributed to these 1:2 species, indicating that both were spirocomplexes in which boron was chelated by two α-diol groups.

In the R_1 complex spectrum, the anomeric carbon at 103 ppm and the CHOH group at 77.2 ppm showed increased $^{1}J_{CH}$ values (ΔJ > 10 Hz), whatever the form of the free ligand. Thus we deduced that the anomeric hydroxyl was chelated and that the second chelated hydroxyl was located at C-2. It excluded that the ligand could be in the β-furanose form, in which C-1 and C-2 would be deshielded by 1 ppm only. If the R_1 complex involved a pyranose form, the variations of chemical shifts (Table 6) would be ≈ 8 ppm at C-1 and ≈ 6 ppm at C-2, but the 79.9 ppm signal would correspond to a $\Delta\delta$ value of ≈ 10 ppm at C-3 or C-4, which is not reasonable. Thus in complex R_1, the ligand had necessarily the α-D-ribofuranose structure, with the boron atom chelated by the C-1,C-2 cis-diol system.

Complex R_2 displayed a ^{13}C NMR spectrum with 3 signals above 80 ppm, including the anomeric carbon signal. Besides, the direct coupling constants of two CHOH groups were increased by nearly 10 Hz. Since only two carbons can be complexed, it implicated that the signal of the C-1 anomeric carbon should not be modified. It excluded that the ligand could be in any pyranose form, in which the anomeric carbon would be strongly deshielded.

The variations of chemical shifts were thus compared (Table 6) for both furanose forms. Since $\Delta J \approx 0$ at C-1, the anomeric carbon must be little deshielded, which would not be possible if the ligand was in α-furanose form. On the contrary, it agreed perfectly with the

assumption of the β-furanose form, showing two equally deshielded carbons at C-2 and C-3. The finding that R_2 was a bis (2,3-β-furanose) chelate was not unprecedented, since the borate complex[18] of adenosine (a D-ribofuranose nucleoside) had a similar structure.

d) complexes of D-xylose and related sugars

Makkee and coll.[15] compared the BL_2^- complexes of D-glucose and D-fructose, but could not find clear signals due to the complex in the ^{13}C spectrum of D-glucose. By analogy with other ligands, they suggested that D-glucose should form a bis (1,2-α-furanose) complex. Pursuing our investigations, we studied the series of D-glucose, D-xylose and L-sorbose. For the three uncomplexed compounds, the ^{13}C spectra show the presence of only the α- and β-pyranose forms at equilibrium (no β-form for L-sorbose). After the addition of borax, we could identify for each compound 5 (pentose) or 6 (hexose) new signals which were attributed to the BL_2^- complexes. The three spectra show striking analogies and probably correspond to complexes of closely related structures.

In the aldose complexes (Table 7), two carbons displayed increased $^1J_{CH}$ values (≈ 176 Hz for C-1 and ≈ 153 Hz for a CHOH group), whatever the assumed form of the free ligand. The complexing α-diol group of aldoses was thus situated at C-1,C-2. For L-sorbose, only one CHOH group showed an increased $^1J_{CH}$ value, because the C-2 anomeric carbon bore no hydrogen. The high δ value obtained for C-2 showed nevertheless that this carbon was deshielded. Since the CH_2OH group at C-1 was obviously not complexed, the α-diol chelating group of L-sorbose was

TABLE 7 - ^{13}C chemical shifts and coupling constants $^1J_{CH}$ of sugars having the xylo configuration and of their BL_2^- borate complexes.

ligand = L	carbon position					
L-sorbose	1	2	3	4	5	6
δ (ppm) in BL_2^- (a)	65.0	111.5	84.5	77.1	81.5	60.9
$^1J_{CH}$ (Hz) in BL_2^-	141	---	153	145.5	144	142.5
δ (ppm) in L(α-fu)	64.3	102.5	77.0	76.2	78.6	61.6
Δδ (ppm)	0.7	9.0	7.5	0.9	2.9	-0.7
δ (ppm) in BL_2^- (b)	65.0	111.5	84.5	81.5	77.1	60.9
δ (ppm) in L(α-py)	64.7	98.3	71.5	74.9	70.6	62.9
Δδ (ppm)	0.3	13.2	13.0	6.6	6.5	-2.0
$^1J_{CH}$ (Hz) in L(α-py)	142	---	142	145	144	144
ΔJ (Hz)	-1	---	11	-1	1.5	-1.5
D-xylose	1	2	3	4	5	
$^1J_{CH}$ (Hz) in BL_2^-	177	156	149.5	145.5	144	
δ (ppm) in BL_2^-	103.4	83.8	77.0	80.5	61.0	
δ (ppm) in L(α-fu)	96.0	77.8	76.2	79.3	61.6	
Δδ (ppm)	7.4	6.0	0.8	1.2	-0.6	
D-glucose	1	2	3	4	5	6
$^1J_{CH}$ (Hz) in BL_2^-	175.5	153	138	141	139.5	141
δ (ppm) in BL_2^-	103.7	83.4	76.9	79.2	69.8	64.9
δ (ppm) in L(α-fu)	97.0	77.7	76.6	78.8	70.7	64.2
Δδ (ppm)	6.7	5.7	0.3	0.4	-0.9	0.7

L-sorbose assignments from ref 21. The spectra of D-xylose and D-glucose were assigned by comparison with those[21] of the corresponding furanosides.
(a) definitive assignment. (b) attempted assignment, found incorrect (see text).

borne by carbons C-2 and C-3.

We excluded that the ligands could be in β-furanose or β-pyranose forms because the complexing hydroxyls would be trans, contrary to all other known borate-sugar complexes which were formed from cis-diol systems. Thus the variations of chemical shifts were calculated for L-sorbose (Table 7) assuming the ligand to be in the α-furanose or α-pyranose forms, but the results did not allow to exclude either hypothesis. In both cases, C-2 and C-3, which were assigned unambiguously, were the most deshielded carbons. In contrast, the assignments of C-4 and C-5 had to be reversed according to the chosen hypothesis, so that their Δδ values could correspond to uncomplexed hydroxyls. In order to get a definitive assignment, we studied the compound forming the more stable complex, L-sorbose, using a 500 MHz spectrometer. The ^{1}H chemical shifts were assigned and related to the ^{13}C signals through a two-dimensional (2D) heterocorrelated experiment. The results[26] showed unambiguously that the 81.5 ppm signal was due to C-5, proving that the ligand was indeed α-L-sorbofuranose.

The assignments for the D-xylose and D-glucose complexes were made by analogy with those for L-sorbose (Table 7), showing analogous variations of chemical shifts. The chemical shifts for the uncomplexed sugars in furanose form were not found in literature and had to be estimated from data[21] on methyl furanosides.

e) complexes of D-lyxose and related sugars

The sugars D-lyxose, D-mannose and D-tagatose formed weak BL_2^- complexes and the ^{13}C spectra of their mixtures with borax showed that much uncomplexed ligand remained. The addition of borax to D-tagatose (initially present as the α-pyranose) caused the appearance of more than six new signals. Two of them, at δ = 111.1 and 103.6 ppm, could be assigned to anomeric carbons, suggesting the formation of two different 1:2 complexes, T_1 and T_2, in nearly equal ratio, as shown by the signal intensities. Because the spectrum of complexed D-tagatose displayed too many signals, all of similar intensities, we decided to make the assignments by comparison with the spectra of the other sugars of the series.

When D-lyxose was complexed, seven new signals appeared in the ^{13}C spectrum, in agreement with the formation of two complexes. Five signals had the highest intensities and were attributed to a prevailing complex (60%) noted L_1 (Table 8). The missing signals of the other complex, L_2 (40%) were admitted to overlap with L_1 signals. Similar results were found with D-mannose, for which six signals were attributed (Table 8) to a main complex M_1 analogous to L_1. We could not resolve the spectrum enough to detect other signals corresponding to a M_2 complex analogous to L_2.

As in the case of D-ribose, the ^{11}B spectra of the three complexed sugars showed only one signal ($\delta \approx -9$ ppm) for the two 1:2 species, which were therefore considered as spirocomplexes in which boron was chelated by two α-diol groups.

The structures of the M_1 and L_1 complexes were determined by remarking that two CHOH groups gave signals with $^{1}J_{CH} \approx 152$ Hz instead of ≈ 142 Hz in any form of the free ligand, and were thus bound to borate. Consequently, the anomeric carbons could not be chelating

TABLE 8 - ^{13}C chemical shifts and coupling constants $^{1}J_{CH}$ of sugars having the lyxo configuration and of their BL_2^- borate complexes.

ligand = L	carbon position					
D-lyxose	1	2	3	4		5
δ (ppm) in complex L_1	103.0	83.5	77.4	82.1		61.6
$^{1}J_{CH}$ (Hz) in complex L_1	173(a)	153	152	142		142
δ (ppm) in L(α-fu)	101.5	77.8	71.9	80.7		61.9
$\Delta\delta$ (ppm)	1.5	5.7	5.5	1.4		-0.3
δ (ppm) in complex L_2	103.0	77.4	69.6	75.7		61.6
$^{1}J_{CH}$ (Hz) in complex L_2	173(a)	152	144	ND		142
δ (ppm) in L(β-fu) (b)	96.3	73.2	71.0	82.1		62.7
$\Delta\delta$ (ppm)	6.7	4.2	1.4	-6.4		-1.1
δ (ppm) in L(α-py)	95.2	71.2	71.7	68.6		64.2
δ (ppm) in L(β-py)	95.2	71.2	73.6	67.7		65.2
D-mannose	1	2	3	4	5	6
δ (ppm) in complex M_1	103.3	83.4	77.0	81.0	75.5	64.6
$^{1}J_{CH}$ (Hz) in complex M_1	173(a)	152	152	144	144	142
δ (ppm) in L(α-fu) (b)	102.7	77.9	72.5	80.5	70.6	64.5
$\Delta\delta$ (ppm)	0.6	5.5	4.5	0.5	-4.9	0.1
δ (ppm) in L(β-fu) (b)	96.6	73.1	71.2[c]	80.7	71.0[c]	64.4
D-tagatose	1	2	3	4	5	6
δ (ppm) in complex T_1	62.3[c]	103.6	83.6	78.0	81.5	61.7[c]
δ (ppm) in L(α-fu)	?	105.7	77.6	71.9	80.0	?
$\Delta\delta$ (ppm)		-2.1	6.0	6.1	1.5	
δ (ppm) in complex T_2	62.3[c]	111.1	78.0	70.6	74.7	61.7[c]
δ (ppm) in L(β-fu)	63.5	103.3	71.7	71.8	80.9	61.9
$\Delta\delta$ (ppm)	-1.2	7.8	6.3	-1.2	-6.2	-0.2

(a) intermediate value for the two overlapping signals.
(b) assignments made from the furanoside spectra[21].
Free ligands assignments from ref 20-24. [c] these assignments may be reversed.

centres, and both pyranose forms of the ligands (in which the C-1 signals appeared at 95 ppm) could be excluded, because the C-1 of the complexes at δ = 103 ppm (which are not bound to boron) would be abnormally deshielded by 7-8 ppm. The same conclusion held for the β-furanose forms of both ligands, in which the C-1 signals were found at $\approx$ 96 ppm. In contrast, the α-furanose forms possessed C-1 signals near 102 ppm, corresponding to $\Delta\delta$ values $\approx$ 0 for the chelation. The corresponding variations of chemical shifts are given in Table 10. The most deshielded carbons, C-2 and C-3, bore a cis-diol group which was the likely chelating site of boron in complexes L_1 and M_1.

Returning now to D-tagatose, the signals of complex T_1 were assigned by analogy with those of L_1 and M_1 (Table 8). The results were in agreement with a 3,4-α-furanose complex.

Our assignments for complexes L_2 and T_2 were guided by the finding of an anomeric carbon signal at δ = 111.1 ppm in the T_2 spectrum. It was the same range as for the complexed anomeric carbons of D-fructose and L-sorbose. Since the assumption of the ligand in pyranose form would imply a very strong and unusual deshielding of this carbon, the only reasonable

choice was to attribute this signal to the complexed anomeric carbon of a tagatofuranose. The second complexed carbon could obviously not be the C-1 CH_2OH group, which was not deshielded, and was thus C-3. Since the chelating α-diol group must be cis, the ligand must be in β-furanose form, in which the C-3 signal appears at 71.7 ppm. The corresponding $\Delta\delta$ value was assumed to be ≈ 8 ppm, as for C-2. Thus the signal of complexed C-3 was expected at $\delta \approx 80$ ppm and we assigned it at 78.0 ppm. This signal had been already attributed to the C-4 of T_1, but was of higher intensity than the other T_1 signals.

It seemed then that, as in the case of D-ribose, two complexes could be formed by the sugars of this series. The T_1, L_1 and M_1 complexes involved the anomeric hydroxyls of the α-furanose ligands and the nearest ring CHOH. The T_2 and L_2 complexes were formed by the β-furanose ligands and did not involve the anomeric hydroxyls, which were trans to the nearest ring CHOH, but they complexed boron by the cis-diol group borne by the furanose ring.

Additional support to this conclusion was given by the $^1J_{CH}$ value, 173 Hz, of the 103 ppm signal of the complexed D-lyxose, which was assumed to be the sum of the overlapping C-1 signals of both complexes. In L_1, C-1 is a complexed carbon and would have a $^1J_{CH}$ value > 175 Hz, as for D-ribose or D-xylose. In L_2, C-1 is not complexed and would exhibit a value of 165-170 Hz, as in most free furanoses. We verified that the decoupled signal was indeed a broadened doublet, as expected if two signals with close $^1J_{CH}$ values overlapped. The same phenomenon was observed in the case of D-mannose.

f) influence of the sugar configuration

It was shown[27] that in polyhydroxy compounds, cis-α-diol groups complexed borate more strongly than any other diol system. This study demonstrated that in all BL_2^- complexes of sugars, borate was bound to two vicinal hydroxyls borne by a furanose ring, in agreement with the earlier result[15] that cis-1,2-cyclopentanediol formed more stable complexes than its C_6 homolog. This effect is so important that the sugars are forced into furanose form by complexation, though they mainly adopt the pyranose structure when uncomplexed. Such a behaviour contrasts with that of inositols[28], which complex borate by axial hydroxyls borne by their cyclohexane rings.

Sugars in which the two ring CHOH groups are trans (series of xylose and arabinose) react with borate by their anomeric hydroxyl groups, which adopt the α or β configuration forming a cis-diol system with the neighbour CHOH. Thus only one complex can be observed. On the other hand, sugars in which the two ring CHOH groups are cis (series of ribose and lyxose) can chelate borate in an additional way implying this diol system. In the latter case, it can be remarked that when the anomeric hydroxyl is cis to the nearest CHOH group (creating thus a cis,cis-triol system) borate only reacts with the α-diol system involving the anomeric OH group. Accordingly, the complexes in which borate is bound to the cis-diol system formed by both ring CHOH groups always possess an anomeric hydroxyl trans to the nearest CHOH group. It illustrates the higher reactivity of the anomeric hydroxyl group compared to that of the CHOH ring groups.

g) structure-stability relationships in borate complexes

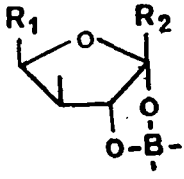
The stability differences between the BL^- complexes are generally small (Table 2) and appear to depend little on the sugar structures. On the contrary, the log β_2 values show large variations, particularly in the pentose series, indicating that the stabilities of the 1:2 complexes are very sensitive to the ligand structures. Previous attempts[10] to relate the complex stabilities to the configurations of the pentoses cannot be further considered, first because most stability constants were of poor accuracy, and second because the pentoses were assumed to react in pyranose form. A representation of the various BL_2^- complexes, grouped in series having the same configuration, is given in Fig. 1. For symmetry reasons, D-sorbose and L-galactose were drawn instead of the actually studied enantiomers.

FIGURE 1 - Structures of the ligands in borate-sugar BL_2^- complexes

1,2-α-D-xylofuranose R_1 = CH_2OH; R_2 = H

1,2-α-D-glucofuranose R_1 = $CHOH-CH_2OH$; R_2 = H

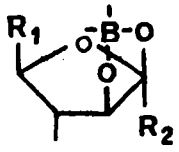
2,3-α-D-sorbofuranose R_1 = R_2 = CH_2OH

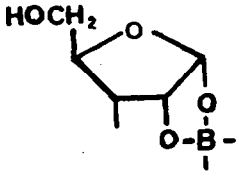


1,2-β-D-arabinofuranose R_1 = CH_2OH; R_2 = H

1,2-α-L-galactofuranose R_1 = $CHOH-CH_2OH$; R_2 = H

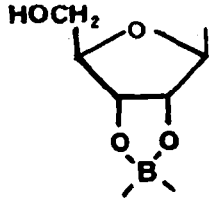
2,3-β-D-fructofuranose R_1 = R_2 = CH_2OH





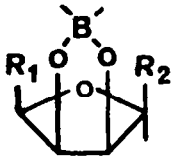
complex R_1

1,2-α-D-ribofuranose



complex R_2

2,3-β-D-ribofuranose

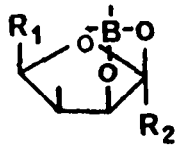


complexes L_1, M_1, T_1

2,3-α-D-lyxofuranose R_1 = CH_2OH; R_2 = H

2,3-α-D-mannofuranose R_1 = $CHOH-CH_2OH$; R_2 = H

3,4-α-D-tagatofuranose R_1 = R_2 = CH_2OH



complexes $L_2, M_2(?), T_2$

1,2-β-D-lyxofuranose

1,2-β-D-mannofuranose

2,3-β-D-tagatofuranose

An important result is that BL_2^- complexes are not, as often postulated, mixtures of interconverting species in which boron is bound to several α or β diol groups of the ligand. Depending on the structure of the chelating sugar, only one or two 1:2 species could be identified in significant amounts. It nevertheless limits the possibilities of discussing the relationships between the complex stabilities and the ligand structure, since the potentiometric method cannot afford the individual constants relative to both species. However, the different 1:2 species were always formed in constant ratio (which is equivalent to a single species), allowing the determination of a mean stability constant for the apparent complexation equilibrium.

The relative stabilities of the complexes can be accounted for by considering the natures of the R_1 and R_2 substituents and their orientations with respect to the borate group. Among sugars of the same type (pentoses, aldohexoses, ketoses) the more stable complexes are those in which the borate ion is trans to both R_1 and R_2 groups (ribose and xylo-gluco-sorbo series). Complexes in which R_1 and the borate ion are cis are weaker (arabino and lyxo series). The nature of the R_2 substituent also has a clear effect, as shown by comparing the ketoses L-sorbose and D-fructose ($R_2 = CH_2OH$) and the related pentoses, D-xylose and D-arabinose ($R_2 = H$), which form complexes of lower stabilities. Thus a hydroxymethyl group borne by the anomeric carbon increases the complex stability when trans to the borate group.

The influence of the nature of the R_1 group is probably the main reason for the large stability differences between the complexes of pentoses and ketoses ($R_1 = CH_2OH$) and the much weaker complexes of aldohexoses ($R_1 = CHOH\text{-}CH_2OH$). A possible interpretation would call upon some sort of steric hindrance by bulky R_1 groups, but must be rejected since :

(i) examination of models revealed that the spiro BL_2^- complexes had "sandwich-like" structures in which the furanose rings were roughly parallel. However, the interval between the corresponding planes appeared too large to allow a steric repulsion between two R_1 groups orientated cis to the borate moiety.

(ii) similar stability differences between complexes of pentoses and hexoses were found in the xylo-gluco series, in which R_1 was trans to the borate moiety, so that no steric interaction between both R_1 groups could be expected.

Besides, the R_1 hydroxyl(s) could interact with the hydroxyls bound to borate when they happened to be in cis orientation. Such an effect was considered to have a destabilizing influence[29] in the case of uncomplexed furanoses. In contrast, the removal of the D-mannose C-6 hydroxyl to give its deoxy derivative, L-rhamnose, strongly decreased the corresponding stability constants, demonstrating that the presence of this non-bonding hydroxyl group could be favourable to complex formation.

In conclusion, the hypothesis[10] that borate complex stabilities were related to the number of possible chelating sites in the ligands was not verified, since some sugars which gave two complexes, i.e. D-lyxose and its series, formed weaker complexes than others,

i.e. D-xylose and its series, which gave one complex only. More generally, the interpretations based on the competitive complexations of α and β diol groups and those considering the axial or equatorial configurations of the chelating hydroxyls in pyranose rings were not supported by our experimental results.

Acknowledgments.- The 500 MHz experiments were made at the Centre de Spectroscopie de l'Université Pierre et Marie Curie (Paris VI) and are gratefully acknowledged.

REFERENCES

[1] J.X. KHYM and L.P. ZILL, J. Am. Chem. Soc., 74, 2090 (1952).
[2] T. OKADA and T. KUWAMOTO, Anal. Chem., 58, 1375 (1986).
[3] J. MORRE and S. MORAZZANI-PELLETIER, C.R. Acad. Sc. Paris, 262, 1729 (1966).
[4] J.F. MENDICINO, J. Am. Chem. Soc., 82, 4975 (1960).
[5] J. KNOECK and J.K. TAYLOR, Anal. Chem., 41, 1730 (1969).
[6] G.L. ROY, A.L. LAFERRIERE and J.O. EDWARDS, J. Inorg. Nucl. Chem., 4, 106 (1957).
[7] W.J. EVANS, E.J. Mc COURTNEY and W.B. CARNEY, Anal. Biochem., 95, 383 (1979).
[8] P.J. ANTIKAINEN and K. TEVANEN, Suom. Kemistil., B 32, 214 (1959).
[9] P.J. ANTIKAINEN and E. HUTTUNEN, Suom. Kemistil., B 46, 184 (1973).
[10] E. HUTTUNEN, Ann. Acad. Sci. Fenn., Ser. A 2, 201, 1 (1984).
[11] J.F. VERCHERE and M. HLAIBI, Polyhedron, 6, 1415 (1987).
[12] J.M. CONNER, J. Inorg. Nucl. Chem., 32, 3545 (1970).
[13] M. VAN DUIN, J.A. PETERS, A.P.G. KIEBOOM and H. VAN BEKKUM, Tetrahedron, 40, 2901 (1984).
[14] M. VAN DUIN, J.A. PETERS, A.P.G. KIEBOOM and H. VAN BEKKUM, Tetrahedron, 41, 3411 (1985).
[15] M. MAKKEE, A.P.G. KIEBOOM and H. VAN BEKKUM, Recl Trav. Chim. Pays-Bas, 104, 230 (1985).
[16] G. BREVARD and P. GRANGER, "Handbook of High Resolution Multinuclear NMR", John Wiley, New-York, 1981, p. 40.
[17] U. WESER, Z. Naturforsch., 22B, 457 (1967).
[18] W. VOELTER, C. BURVENICH and E. BREITMAIER, Angew. Chem. Int. Ed. Engl., 11, 539 (1972).
[19] H. PELMORE and M.C.R. SYMONS, Carbohydr. Res., 155, 206 (1986).
[20] E. BREITMAIER and W. VOELTER, "^{13}C NMR Spectroscopy", 2nd ed., Verlag Chemie, Weinheim, 1978.
[21] K. BOCK and C. PEDERSEN, Adv. Carbohydr. Chem. Biochem., 41, 27 (1983).
[22] P.E. PFEFFER, K.M. VALENTINE and F.W. PARRISH, J. Am. Chem. Soc., 101, 1265 (1979).
[23] J. REUBEN, J. Am. Chem. Soc., 106, 6180 (1984).
[24] M.J. KING-MORRIS and A.S. SERIANNI, J. Am. Chem. Soc., 109, 3501 (1987).
[25] H. ASAOKA, J. Chem. Soc. Perkin Trans. II, 1499 (1983).
[26] D. DAVOUST, S. CHAPELLE and J.F. VERCHERE, unpublished results.
[27] R.E. MOORE, J.J. BARCHI and G. BARTOLINI, J. Org. Chem., 50, 374 (1985).
[28] C.F. BELL, R.D. BEAUCHAMP et E.L. SHORT, Carbohydr. Res., 147, 191 (1986).
[29] S.J. ANGYAL, Angew. Chem. Int. Ed. Engl., 8, 159 (1969).