Boronic acids as fructose sensors. Structure determination of the complexes involved using $^1J_{CC}$ coupling constants

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Complex formation between *p*-tolylboronic acid and D-fructose was investigated by ^{13}C NMR spectroscopy both in neutral nonaqueous and alkaline aqueous solutions to evaluate the possibilities of boronic acid based fructose sensors. Under both conditions a mixture of complexes was observed. The structures of the complexes in solution were assigned on the basis of $^1J_{CC}$ coupling constants which provided the information of the binding sites for the formed cyclic boronic esters. In alkaline aqueous solution seven different complexes were observed. At a 1:1 boronic acid: fructose ratio the major complex (82%) was β-D-fructofuranose 2,3,6-tri-*O*-(*p*-tolylorthoboronate) 1. The minor complex was β-D-fructofuranose 2,3-(*p*-tolylhydroxyboronate) present in two diastereomeric forms 2a and 2b. At higher boronic acid: fructose ratios four additional complexes were observed namely the four possible diastereomeric forms of β-D-fructopyranose 2,3:4,5-bis(*p*-tolylhydroxyboronate) 3a–d. Under nonaqueous conditions signals arising from 13 different compounds were observed. Five of these complexes were structurally assigned. At a 1:1 boronic acid: fructose ratio the major complex was β-D-fructofuranose 2,3-(*p*-tolylboronate) 5 present in 46%. At a ratio of 4:1 the major species was β-D-fructopyranose 2,3:4,5-bis(*p*-tolylboronate) 7 present in 67%. Two additional monoesterified pyranoses and one furanose were observed. They were assigned to the β-D-fructopyranose1,2-(*p*-tolylboronate) 8, the α-D-fructopyranose 1,3-(*p*-tolylboronate) 9 and the β-D-fructofuranose 1,3-(*p*-tolylboronate) 6. It is concluded that results of earlier studies on binding constants bear no relation to the actual formed complexes.

Introduction

Complexes between boronic acids and polyhydroxy compounds have been studied intensively since the early work of Böeseken.[1] From 1952 to around 1965, Torssell[2] published a series of studies of aromatic boronic acids, which included equilibria between the boronic acids and different polyols, their inhibitory effects on enzymes, effects on cell walls, cell growth *etc.* Since then, the research on boronic acids has developed into a still more exciting field. Today more complex boronic acids are recognized as potential and selective saccharide sensors,[3] selective membrane transport molecules[4] and effective enzyme inhibitors.[5]

The key action of boronic acids is the reversible formation of cyclic esters with diols, from here on denoted as complexes.[6] Boric and boronic acid complexation with a large range of polyols in aqueous solution has been evaluated by measurements of the equilibrium constants. These have been determined by a number of methods including pH depression,[7] potentiometric titration,[8] calorimetry,[9] polarimetry,[10] circular dichroism,[3d,10,11] fluorescence,[3a,3c,3f,12] absorption[13] and voltametry.[14] A general finding is that D-fructose, compared to D-glucose, has a very high observed association constant to both borate and monodentate arylboronates in aqueous solution. For borate mono-, di- and spiro-complexes of fructose have been reported.[7a,10a,15] For boronic acids there is only one report dealing with the structures of these evidently strong fructose complexes[16] although the structures were not determined. From nonaqueous solution β-D-fructopyranose 2,3:4,5-bis(phenylboronate)[17] and the analogous bis(4-vinylphenylboronate)[18] have been isolated in the crystalline state.

To gain more insight into which structural features are important for strong complex formation, and to evaluate the possibility of specific boronic acid based fructose sensors, we decided to investigate the structure of the complexes formed between D-fructose and *p*-tolylboronic acid. In our recent work we showed that $^1J_{CC}$ coupling constants together with the standard information from ^1H and ^{13}C NMR spectroscopy allowed the assignment of the binding sites for the complexes formed between D-glucose and two different boronic acids.[19] Extending this methodology with regard to $^1J_{CC}$ we here disclose the solution structures of the complexes formed between *p*-tolylboronic acid and D-fructose under both neutral nonaqueous and alkaline aqueous conditions. The equilibrium mixtures showed a much higher degree of complexity than previously reported.[16]

Results

The interaction between *p*-tolylboronic acid and D-fructose was studied both in alkaline aqueous (pD *ca.* 11) and in neutral nonaqueous solutions [$(CD_3)_2SO$] with varying boronic acid: fructose stoichiometric ratios (1:1, 2:1 and 4:1). In aqueous solution at a ratio of 1:1 signals corresponding to three complexes named **1**, **2a** and **2b** in the ratio 82:13:5 appeared in the ^{13}C NMR spectrum of the equilibrated mixture. When the amount of *p*-tolylboronic acid was increased to a ratio of 2:1, signals from four new complexes **3a–3b** appeared. At even higher ratios (4:1) the lines of the complexes broadened and only two sets of carbohydrate signals remained observable. One set with a $\Delta\nu_{\frac{1}{2}}$ *ca.* 10 Hz corresponded closely to **1**. The other set had signals at the mean values of **3a** and **3b** with $\Delta\nu_{\frac{1}{2}}$ varying between 5 and 100 Hz in accordance with intermediate fast exchange between **3a** and **3b**. Whether this exchange also included the complexes in minor amounts, namely **3c** and **3d**, could not be concluded. The relative proportions of the complexes formed at the different stoichiometric ratios are given in Table 1. No signals corresponding to free fructose were

Table 1 Proportions (%) of fructose complexes with *p*-tolylboronic acid in aqueous alkaline solution. Equilibrium mixtures at different boronic acid : fructose ratios

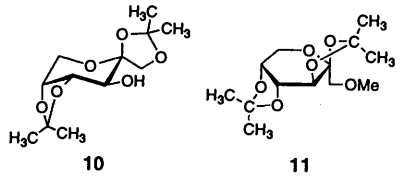
Compound	1:1	2:1	4:1
1	82	48	*ca.* 40[a]
2a	13	7	
2b	5	3	
3a		17	*ca.* 60[a,b]
3b		15	
3c		5	
3d		4	

[a] Broad signals. [b] Average signals from $(\mathbf{3a})_2$- and $(\mathbf{3b})_2$-D-fructose.

observed, and the amount of the individual fructose isomers was estimated to be less than 0.5%. The reaction between fructose and boric acid at a stoichiometric ratio of 1:3 and pD = 11–12 was also studied. Signals from several complexes were observed with one major component named **4**.

In $(CD_3)_2SO$ solution signals corresponding to five different complexes named **5**–**9** appeared in the ^{13}C NMR spectra of the equilibrated mixtures at varying boronic acid: fructose ratios. The ratios of the complexes formed, including the proportions of free fructose isomers present, are given in Table 2. The 1:1 stoichiometric solution contained eight further complexes in minor amounts (<2%). Only the C-2 carbon signals were clearly visible. The chemical shifts (ppm) and $^1J_{CC}$ coupling constants (Hz) given in parentheses measured for these C-2 carbon atoms were: 213.1 (42, 42); 212.7 (42. 42); 209.0 (46, 42); 113.2 (45, 47); 110.2 (45, 47); 96.5 (52, 47); 95.9 (54.9, 45.0); 91.2 (42.1, 48.7).

The assigned ^{13}C chemical shifts for the fructose part of the complexes studied are compiled in Table 3. Table 4 contains the measured $^1J_{CC}$ coupling constants. The tables also include values measured for the five fructose isomers together with data for the model compounds **10**–**13**. The $^1J_{CC}$ coupling constants



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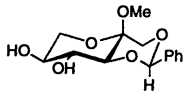

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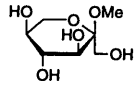

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in the model compounds were measured in natural abundance using the INADEQUATE technique. The remaining $^1J_{CC}$ values were obtained from ^{13}C NMR spectra of samples prepared with uniformly $^{13}C_6$ labelled fructose. The assignments followed directly from the $^1J_{CC}$ coupling information. The couplings over longer distances were small compared to the one bond coupling constants and did not interfere in the interpretation of the spectra. This assignment procedure is unequivocal except in cases where carbon atoms have very similar $^1J_{CC}$ coupling constants. This was found for C-3 and C-4 in **1** and C-3, C-4 and C-5 in **2a**. For both of these complexes the H-3 doublet (*J* 1.3 and 1.8 Hz for **1** and **2a**, respectively) was resolved in the ^1H NMR spectra and correlated to C-3 using ^1H–^{13}C heterocorrelated spectra. In **2a** C-4 was assigned using the fact that second-order coupling effects between the two close lying ^{13}C signals at 88.4 and 86.7 (C-3) ppm were absent. Therefore the former signal was assigned to C-5, and consequently the signal at 80.2 ppm to C-4. In complex **5** the absence of second-order effects between the signals at 87.7 and 88.0 ppm means

Table 2 Proportions (%)[a] of fructose *p*-tolylboronic acid complexes and free fructose isomers in $(CD_3)_2SO$. Equilibrium mixtures at different boronic acid : fructose ratios

Compound	1:1	2:1	4:1
5	46	47	28
6	5	5	2
7	6	33	67
8	8	5	2
9	4	3	1
β-D-Fructopyranose	7		
β-D-Fructofuranose	11	2	
α-D-Fructofuranose	5		

[a] Relative to total amount of complexed and free fructose.

that C-4 can be assigned only to the signal at 76.3 ppm. The assignments of C-4 and C-5 in **6** are based solely on chemical shift considerations.

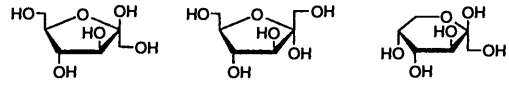
In the fructoketose isomer (Fig. 1) the signal at 66.2 ppm showed a 12.2 Hz coupling besides one $^1J_{CC}$ coupling of 40.4 Hz. In ketones a large $^2J_{CC}$ is usually found between the two α-carbon atoms of the keto group,[20] and the 12.2 Hz coupling therefore assigns the signal as C-1. Due to overlapping signals, couplings for C-3 and C-6 were not resolved.

The complexes named **2a** and **2b** had almost identical ^{13}C NMR parameters as was also the case for the complexes named **3a** to **3d**. Limited coupling information was obtained for the complexes **2b**, **3c** and **3d**, all present in minor amounts. The assignment of **2b** is made in analogy with that of **2a** and the assignments of **3c** and **3d** follow those of **3a** and **3b**. ^{13}C Chemical shifts have been reported for compound **10**[21] and for the fructose isomers.[16,22] The assignments obtained in this work agree with those reported. For α-fructopyranose, present in minor amounts, our chemical shift values differ from those reported.[22b]

Discussion

Fructose exists in solution in a complex mutarotational equilibrium between the five isomeric forms shown in Fig. 1. The measured ratios of the isomers present at equilibrium in D_2O and $(CD_3)_2SO$ solutions at 25 °C are very different and given below the structures. In D_2O, only four of the five isomers have been reported within the same experiment.[15,23] Depending upon the NMR nuclei studied, either the acyclic keto or the α-pyranose form was not detected. In Me_2SO only the proportions of the four cyclic structures have been reported.[23a]

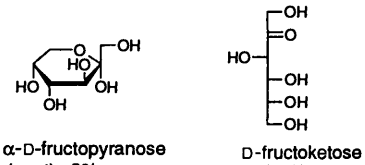
Discrimination between the pyranose, furanose and acyclic keto form of fructose present in each of the complexes **1**–**9** was done on the basis of the chemical shift values. Furanose rings show substantially higher chemical shift values of the ring carbon atoms compared to pyranose rings.[15a] Compounds **1**, **2a**, **2b**, **5** and **6** clearly have chemical shifts in agreement with a



β-D-fructofuranose α-D-fructofuranose β-D-fructopyranose

D_2O (neut) 23% 6% 68%

$(CD_3)_2SO$ 47% 19% 26%



α-D-fructopyranose D-fructoketose

D_2O (neut) 2% 0.7%

$(CD_3)_2SO$ 4% 4%

Fig. 1 Structures and equilibria ratios (%) of the fructose isomers present in D_2O and $(CD_3)_2SO$

Table 3 ^{13}C Chemical shifts (ppm) for the fructose part of *p*-tolylboronic and boric acid complexes and model compounds

Compound	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Fructofuranose[a]	65.5	104.3	78.3	77.3	83.5	65.2
1[b]	66.1	113.4	84.7	82.1	87.6	67.9
2a[b]	67.4	114.7	86.7	80.2	88.4	65.0
2b[b]	67.3	114.6	87.7	80.4	88.0	64.6
β-D-Fructopyranose[a]	66.7	100.9	70.4	72.5	72.0	66.2
3a[b]	69.6	104.6	75.9	72.5	71.5	67.1
3b[b]	69.3	104.2	76.0	73.8	71.4	66.6
3c[b]	69.6[c]	103.5	76.8	72.3*	72.0*	66.8
3d[b]	69.2	103.1	76.6	74.3	71.8	66.3
4[b]	69.4	103.2	75.3	72.6	70.9	66.6
β-D-Fructofuranose[c]	62.8	101.8	75.2*	75.6*	81.8	62.8
5[c]	63.0	115.2	87.7*	76.3	88.0*	61.5
α-D-Fructofuranose[c]	63.6	104.0	82.8	75.7	80.8	61.0
6[c]	64.1	99.8	82.3	78.1	85.1	62.1
β-D-Fructopyranose[c]	64.3	97.9	67.7	69.8*	69.0*	62.9
7[c]	64.0	105.0	72.1*	71.9*	71.4*	61.0
8[c]	72.9	107.6	69.6*	69.0*	68.8*	65.5
α-D-Fructopyranose[c]	63.7	97.1	68.0	71.4	62.9	58.7
9[c]	66.5	92.6	*d*	67.3	70.2	59.7
α-D-Fructofuranose[a]	65.8	107.2	84.8	78.9	84.1	63.2
D-Fructoketose[c]	66.2	213.5	75.4	72.3*	70.5*	63.7
10[c]	70.8	105.0	68.7	76.7	72.9	59.5
11[c]	75.0	103.7	71.0	70.9	71.8	61.7
12[c]	66.2	92.4	81.5	69.8	70.4	63.2
13[c]	60.9	99.8	71.9	73.9	69.8	62.6

[a] In D_2O. [b] In D_2O at pD = 11–12. [c] In $(CD_3)_2SO$. [d] Overlapping signals. * Assignments can be interchanged.

Table 4 $^1J_{CC}$ coupling constants (in Hz) for the fructose part of *p*-tolylboronic and boric acid complexes and model compounds.[d]

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
β-D-Fructofuranose[a]	51.0	44.2	40.3	38.9	41.6
1[b]	54.1	37.4	40.3	36.9	36.3
2a[b]	53.4	37.5	41.1	38.2	41.2
2b[b]	53.7	37			41.6
5[c]	53.8	37.0	41.5	37.9	42.0
α-D-Fructofuranose[c]	50.4	47.3	40	40	42.7
6[c]	49.6	43	43	38.4	42.7
β-D-Fructopyranose[a]	49.8	46.9	37.8	37.5	35.4
3a[b]	51.7	38.5	44.3	34.1	38.9
3b[b]	51.5	38.8	44.3	34.0	38.9
3c[b]	51	38			
3d[b]	51	38			
4[b]	52.6	37.4	44.3	33.8	39.5
7[c]	53.0	35.1			39
8[c]	42.7	48.9			36.6
α-D-Fructopyranose[c]	51.9	46.5	38.5	38.5	40.4
9[c]	48.1	44.3	41.2	37.4	40.1
D-Fructoketose[c]	40.4	45.0	40	43	43
10[c]	43.3	49.4	40.9	32.4	39.7
11[c]	55.8	38.5		33.0	40.3
12[c]	49.0	45.7	40.9	39.1	38.9
13[c]	51.1	48.5	38.4	37.9	39.7

[a] In D_2O. [b] In D_2O at pD = 11–12. [c] In $(CD_3)_2SO$. [d] The absence of an entry indicates that the value has not been measured either due to overlapping signals or too strongly coupled nuclei.

five-membered fructose ring, and **3a–3d**, **4** and **7–9** have values in accordance with complexation of fructose in the pyranose form (Table 3).

Recently, we have shown that exceptionally low values for $^1J_{CC}$ are measured for vicinal diols when the O–C–C–O fragment becomes incorporated in a five-membered ring, as is the case for vicinal cyclic boronic esters.[19] Otherwise, the pattern of measured $^1J_{CC}$ in aldofuranoses and aldopyranoses is remarkably constant and variations with stereochemistry are reported to be within a few Hz.[24] To examine the relationship further we have measured $^1J_{CC}$ for all the carbon atoms of a series of cyclic acetals and orthoester derivatives of carbohydrates.[25] In this series we allowed variation not only of the C–C but also of the C–O torsional angles in R–O–C–C–O–R′ fragments. For a particular fragment $^1J_{CC}$ covered a range of 17 Hz. The general finding was that the C–O torsional angles and thereby the orientation of the oxygen lone pairs are the most important parameters in determining the size of $^1J_{CC}$. Variations of C–C torsional angles also influence $^1J_{CC}$ but to a minor degree. For both torsional angles, the $^1J_{CC}$ coupling constant reaches a maximum for the *anti* and a minimum for the eclipsed configuration. These results agree with the calculations of Serianni and co-workers on torsional effects on the $^1J_{CC}$ coupling constant in ethylene glycol.[26] Thus, formation of cyclic acetals leads to a decrease of $^1J_{CC}$ within the 1,3-dioxolane ring and a concurrent increase of $^1J_{CC}$ at neighbouring C–C fragments outside the ring. This reflects the change in orientation of the oxygen lone pairs relative to the relevant C–C bonds. The measured values of $^1J_{CC}$ of the model compounds **10–13** are compiled in Table 4. A large decrease in $^1J_{C1-C2}$ (6.5) and $^1J_{C4-C5}$ (5.1) in **10** and $^1J_{C2-C3}$ (8.4) and $^1J_{C4-C5}$ (4.5) in **11**, compared to the free pyranoses, is seen upon formation of the five-membered cyclic acetals containing these carbon fragments. The 1,3-acetal of **12** is a six-membered ring and both carbon fragments involved in the ring show a decrease in $^1J_{CC}$ (2.1 and 2.8) relative to compound **13** as a reference, but the decrease is significantly smaller than seen in the five-membered rings of **10** and **11**. An increase at the neighbouring fragments outside the dioxolane ring when compared to values for the appropriate uncyclized compounds are observed for $^1J_{C2-C3}$ (2.5), $^1J_{C3-C4}$ (3.1) and $^1J_{C5-C6}$ (4.3) in **10**, $^1J_{C1-C2}$ (6.0) and $^1J_{C5-C6}$ (4.9) in **11** and $^1J_{C3-C4}$ (2.5) in **12**. In conclusion, the position of ring formation can be detected by measurements of $^1J_{CC}$, and this information has been applied in the structural assignments of the complexes **1–9** (see below).

Complexes **1–4** were formed in alkaline aqueous solution at pD = 11–12. Under these conditions the boron atom is negatively charged and tetrahedral. The fructofuranose complex **1** has a very low value for $^1J_{C2-C3}$. This points to a cyclic boronic ester with a *cis* 2.3-bonding and therefore to a β-furanose complex. The value for $^1J_{C5-C6}$ is however also very low, indicating that complexation also involves C-6 in such a way that the B–O–C-6–C-5 dihedral angle decreases. Complexation at positions 4,6 is impossible for geometric reasons. Boronic acid complexation at positions 2,3,6 results in a B–O–C-6–C-5 torsional angle of *ca.* 60° together with a large decrease of the O–C-5–C-6–O dihedral angle. Complex **1** is therefore assigned the structure β-D-fructofuranose 2,3.6-tri-*O*-(*p*-tolylorthoboronate), as shown in Fig. 2.

The complexes **2a** and **2b** are also furanoses but in contrast with **1** only $^1J_{C2-C3}$ shows the low value. As the remaining $^1J_{CC}$ coupling constants in the ring of **2a** are almost unchanged except for the expected increase seen for C1-C2 and C3-C4 fragments following a complexation at the 2.3-position, we conclude the structure of **2a** to be β-D-fructofuranose 2.3-(*p*-tolylhydroxyboronate). Two diastereomers of this structure are *a priori* possible as the boron atom provides an additional stereocentre.[4c,19] The complexes **2a** and **2b** have almost identical ^{13}C NMR spectra. Complex **2b** was present in the equilibrated solution in minor amounts (see Table 1) and $^1J_{C3-C4}$ and $^1J_{C4-C5}$ could not be measured due to overlapping signals. We suggest **2a** and **2b** to be the *endo* and *exo* diastereomers shown in Fig. 2.

The four complexes **3a–3d** have identical $^1J_{CC}$ values within experimental error and only slightly different ^{13}C NMR chemical shifts. However, only $^1J_{C1-C2}$ and $^1J_{C2-C3}$ were measurable for **3c** and **3d** present in small amounts. The complexes are pyranoses with low $^1J_{C2-C3}$ and $^1J_{C4-C5}$ values. We have assigned **3a–3d** to be the four diastereomers of β-D-fructopyranose 2,3 : 4,5-bis-(*p*-tolylhydroxyboronate) (Fig. 2). These four diastereomers, implied by the two boron stereocentres, are expected to show very similar NMR spectra for the carbohydrate ring carbon atoms.

In a recent study by van den Berg *et al.*[15a] of the borate ester formation of saccharides they proposed the formation of a

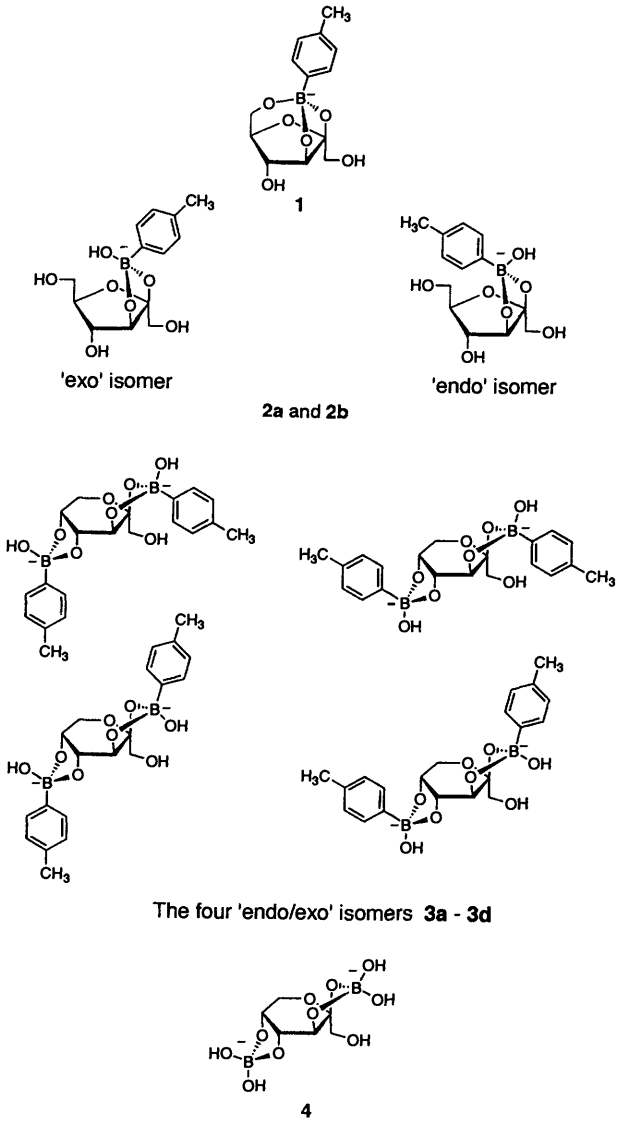


Fig. 2 Structures of the *p*-tolylboronic and boric acid complexes formed with fructose in aqueous alkaline solution

1,2,3:4,5-diborate ester of β-D-fructopyranose in alkaline aqueous solution on the basis of ^{13}C NMR chemical shifts. The chemical shifts given corresponded very closely to those of compound **3**. We have therefore measured the one-bond C–C coupling constants (see Table 4) of the complex. The $^1J_{CC}$ coupling constants are very close to those of **3a** and **3b** and the large value of $^1J_{C1-C2}$ 52.6 excludes C-1 as a binding site. We therefore reassign the structure to the 2,3:4,5 diborate complex **4**.

The data in Table 1, obtained under alkaline aqueous conditions, show that the amount of the complexes **1**, **2a**, **2b** decreased by increasing the boronic acid:fructose stoichiometric ratios, opposite to that found for **3a–3d**. This points to **1**, **2a** and **2b** being mono- and **3a–3d** di-boronic acid complexes in accordance with the structures proposed.

In neutral nonaqueous solution the existence of 13 different complexes was evident from the ^{13}C NMR spectra (see Results). Three of these were complexes of the acyclic keto form of fructose. At a stoichiometric ratio of 1 : 1 three chemical shifts at 213.1, 212.7 and 209.0 ppm, assignable to C-2 in open chain keto forms, were observed. Two of these chemical shifts are very close to C-2 for the free fructoketose, but the measured $^1J_{C1-C2}$ and $^1J_{C2-C3}$ values are clearly different (see Results). These results indicate the presence of three *p*-tolylboronic acid complexes of the open chain keto form of fructose in solution.

Of the last ten observed complexes one, denoted **5**, was present in larger amounts. It was a furanose and the ^1H NMR

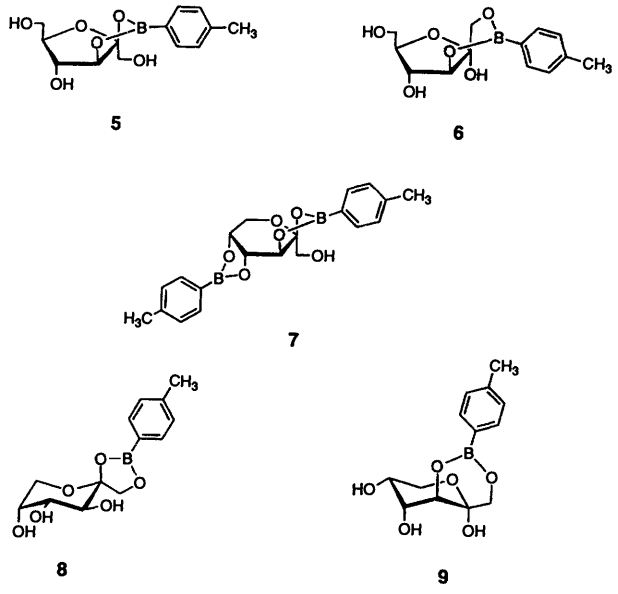


Fig. 3 Structures of *p*-tolylboronic acid complexes formed with fructose in $(CD_3)_2SO$

spectrum showed the presence of three hydroxy groups as one doublet, one triplet and an unresolved broad signal. The $^1J_{CC}$ values show the same characteristics as those of **2a**, and accordingly we have assigned **5** as the β-D-fructofuranose-2,3-(*p*-tolylboronate) (Fig. 3). Complex **6**, also being a furanose has been assigned the α-D-fructofuranose-1.3-(*p*-tolylboronate) (Fig. 3). The chemical shift value for C-2 in **6** is 13–15 ppm lower than those of the furanose complexes **1**, **2** and **5** all complexed at the C-2 position. Only smaller changes in the $^1J_{CC}$ coupling constants, compared to the free α- and β-fructofuranose, were observed. This is in accordance with a six-membered 1,3,2-dioxaborinane ring analogous to the 1,3-acetal ring in **12**. We conclude the binding sites of structure **6** to be 1,3 of the α-D-furanose form. Binding to the β-isomer is geometrically impossible, when incorporating a trigonal planar boron atom.

Complex **7** is a pyranose derivative. The very close chemical shifts of C-3, C-4 and C-5 formed a strongly coupled system with overlapping signals which prohibited the observation of $^1J_{C3-C4}$ and $^1J_{C4-C5}$. The $^1J_{C2-C3}$ is very low and points to a 2,3-bonding and therefore a β-pyranose. The value for $^1J_{C5-C6}$ shows an increase (3.6) as expected for additional complexation at the 4,5-position. The relative amount of **7** increases at higher boronic acid:fructose ratios (see Table 2) indicating **7** to be a diboronic acid complex. In agreement with these observations we determine the structure of **7** to be β-D-fructopyranose 2,3:4,5-bis(*p*-tolylboronate).

For the reasons given above, $^1J_{C3-C4}$ and $^1J_{C4-C5}$ were not measured for the pyranose complex **8**. The measured coupling constants show a very low value for $^1J_{C1-C2}$ and a small increase for $^1J_{C2-C3}$ relative to both α- and β-fructopyranose and this suggests complexation at the 1,2-position. These two values for $^1J_{CC}$ agree well with those of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose. **10**. The value measured for $^1J_{C5-C6}$ in **8** does not agree with $^1J_{C5-C6}$ in **10** which is also bound in the 4,5-position, but it relates to the value of the free β-fructopyranose. The possibility of **8** being an α-isomer is excluded due to its significantly lower value of $^1J_{C5-C6}$ as compared to the free α-D-fructopyranose.† Complexing also at the 4,5-position is

† The difference of 5 Hz in $^1J_{C5-C6}$ between α- and β-D-fructopyranoses can be evaluated by their conformational differences. The most likely conformation for a 1,2 complexed α-D-fructopyranose is $^5C_2$. Here the O-5–C-5–C-6–O-6 torsional angle is approximately 180°. whereas the probable $^2C_5$ conformation for a similar β-D-fructopyranose complex gives a torsional angle of *ca.* 60°. This explains the lower values of $^1J_{C5-C6}$ found in β-D-fructopyranoses.

expected only to increase the value further because of a resulting reduction of the H–O-5–C-5–C-6 torsional angle. The amount of **8** decreases at higher boronic acid:fructose ratios in agreement with **8** being a mono-boronic acid complex. These results point to **8** being the β-D-fructopyranose 1,2-(*p*-tolylboronate) as shown.

The pyranose complex **9** is only present in small amounts. The variation with the stoichiometric ratio (see Table 2) suggests this complex to be a monoboronate. The two $^1J_{C,C}$ coupling constants around the C-2 carbon atom are both reduced and the values are very similar to those measured for complex **6** and model compound **12**, both being bound in positions 1 and 3. Furthermore, the chemical shift measured for C-2 in **9** is shifted upfield relative to C-2 in the free fructopyranoses. These results determine **9** to be complexed in the 1,3-position. The size of $^1J_{C4\text{-}C5}$ in **9** is unchanged relative to both α- and β-fructopyranose and exclude further binding sites in agreement with a monoboronate complex. Unlike the 1,3-bound furanose complex **6**, the pyranose form of fructose allows the existence of both an α- and β-isomer. $^1J_{C5\text{-}C6}$ values differ by as much as 5 Hz in α-relative to β-fructopyranose. Complex **9** has a value for this coupling constant very close to that of α-fructofuranose and on this basis we assign **9** the α-D-fructopyranose 1,3-(*p*-tolylboronate) structure shown.

The above account of the intricate mixture of complexes leads to the result that earlier studies on binding constants, with their lack of knowledge of the stoichiometry and the proportions of the complexes formed, show no relationship between the physical measurements and the complexes formed. Additionally, the observed binding constants for different sugars cannot be correlated with complex stabilities without accounting for differences in the anomeric equilibria. The present study showed that *p*-tolylboronic acid in a 1:1 mixture binds preferably to β-fructofuranose which is present to an extent of 23% in equilibrated aqueous solution. In contrast glucose, which was generally believed to form a weaker complex, binds only in the less abundant α-glucofuranose form.[19] This anomer is present in water only at 0.14%.[27] Accounting for the anomeric equilibrium constants the 'local' association constants can be calculated as recently done by van den Berg *et al.*[15a] in the borate case. They found a higher 'local' binding constant of borate to the α-glucofuranose than to the β-fructofuranose. The tridentate β-furanose complex was found only to be formed at high pH, which means that it is not generally a more stable structure than the 2,3-β-furanose complex.

Conclusions

In this study we have presented evidence for the very complex equilibrium between D-fructose and *p*-tolylboronic acid. By use of ^{13}C NMR and especially $^1J_{CC}$ coupling constants we have been able to assign complexes found in only a few percent in solution.

For selective sensing of carbohydrates several boronic and diboronic acids have been suggested.[3a,3d,3f,12,28] In one case structure optimization caused a selectivity for glucose in the presence of a physiological fructose level,[28] although in most cases the response to fructose clearly exceeded that of other sugars. In the search for new selective boronic acid based sensors with a high affinity for fructose one cannot evaluate the structure-binding efficiency without a careful study of the complexes involved. In addition, the readout of sensors normally relies on segregated species responsible for a discrete measurable property, and therefore a more simple and selective binding is required. We believe that our results reveal the possibility of designing a selective diboronic acid based sensor to complex the β-D-fructopyranose which is the major anomer present in neutral aqueous solution.

Experimental

Methods

Samples for NMR were prepared as previously described.[19] NMR spectra were obtained at 25 °C. on a Varian Unity 400 NMR spectrometer ^{13}C (100 MHz) and ^1H (400 MHz). Internal $SiMe_4$ for nonaqueous and DSS for aqueous solutions; chemical shifts are in ppm. The ^{13}C NMR spectra of aqueous solutions were also obtained at 4 °C. This resulted, for some carbon atoms, in separation of otherwise overlapping coupling patterns. Due to extensive peak overlap the ^1H NMR spectra were not analysed except in special cases. Spectra were recorded with sufficient delay time between pulses to obtain reliable signal intensities. This was important both in the process of sorting out signals belonging to the various structures and to calculate the values given in Scheme 1 and Tables 1 and 2. The solutions for measuring $^1J_{CC}$ by the INADEQUATE technique were 0.3 M. Samples applying uniformly $^{13}C_6$ labelled D-fructose were 0.1 M and other samples 0.2 M in fructose.

Materials

Uniformly $^{13}C_6$ labelled D-fructose was purchased from Cambridge Isotope Laboratories. *p*-Tolylboronic acid was prepared as described previously.[19] Compounds **10** and **11** were prepared by the method of Brady.[29] Compounds **12**[30] and **13**[31] were prepared according to the cited literature.

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