N.m.r. studies of complexes formed by D-fructose and borate ions in aqueous solution

HEATHER PELMORE AND MARTIN C. R. SYMONS
Department of Chemistry, The University, Leicester LE1 7RH (Great Britain)
(Received October 3rd, 1985; accepted for publication, May 16th, 1986)

The interaction of borate ions and hydroxy compounds is well established[1,2] and complexes involving 1,2-diols are generally more stable than those with 1,3-diols[3]. Kennedy *et al.*[4] suggested that chemical shifts of the ^{11}B resonances of sugar complexes can be used to determine the size of the ring in which boron is involved. The results were interpreted for D-glucose in terms of complexes involving 1,2-furanose and 1,2-pyranose structures. Gorin *et al.*[5] claimed that ^{13}C-n.m.r. data (chemical shifts and width changes) can be used to indicate the hydroxyl groups involved in complex formation.

We have introduced[6] a different approach to the study of the formation of complexes of carbohydrates with cations and anions, which utilises the fact that well-resolved ^{1}H resonances for hydroxyl protons can be observed directly using low temperatures and careful control of pH. The changes in these resonances give more direct measures of the involvement of these groups in complex formation.

We now report a comparative study of a sugar–borate complex using changes in both $^{1}H(OH)$ and ^{13}C resonances.

D-Fructose (Fig. 1)[7] was selected for study because of the industrial importance of D-fructose–borate complexes[8]. These complexes are utilised in the preparation of D-fructose from solutions of D-glucose since they switch the initial equilibrium almost completely in favour of D-fructose. Although sodium tetraborate was used, this hydrolyses in dilute aqueous solution[9,10] to give high concentrations of BO_4^{3-} which are thought to be responsible for complex formation, and this is assumed in the sequel.

The resonances for anomeric OH protons occur[6] on the low-field side of the non-anomeric OH proton resonances which, in turn, are on the low-field side of the water resonance. This situation is illustrated in the typical spectrum given in Fig. 2.

Anomeric hydroxyl protons. — Addition of aqueous solutions of sodium tetraborate to aqueous D-fructose resulted in a reduction in the anomeric hydroxyl proton resonances (*β-p* and *β-f*). A plot of the normalised sum of the anomeric proton peak areas against the increase in borate concentration is given in Fig. 3. The important aspects of such plots are linearity of the curves and the fact that the

0008-6215/86/$ 03.50

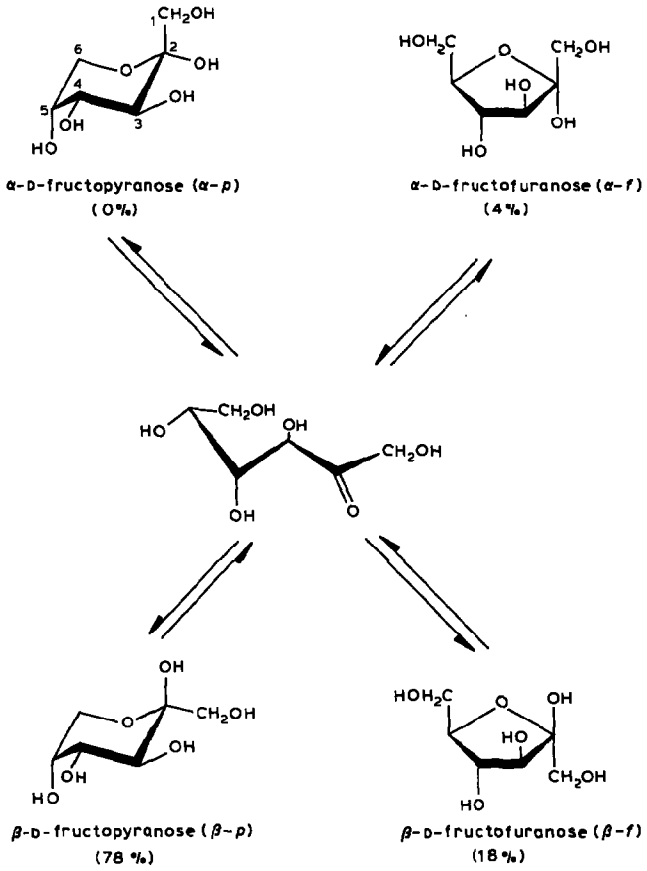

Fig. 1. Tautomeric equilibria[7] of D-fructose (20%) in D_2O at 0°.

slopes are close to 2.0. These results show that the equilibrium strongly favours complex formation, at least in the region of pH 6. Previous studies using optical rotation suggested complexation was complete at pH >7 for more dilute solutions[11].

Non-anomeric hydroxyl protons. —The narrowest peaks for non-anomeric hydroxyl protons were obtained at pH 7.2. The four protons gave rise to a single broad resonance which was not split even at 300 MHz.

Addition of borate caused a reduction in the area of the composite band, together with the growth of a new band downfield therefrom. There was a 75% loss in intensity of the original band on complete complex formation. This means either that two non-anomeric hydroxyl groups are involved in complexing or that one is so involved and that another has had its resonance shifted upfield, so that it is concealed by the intense band for water. Since it is unlikely that boron is hexacoordinated, the latter explanation is favoured. Thus, of the four non-anomeric

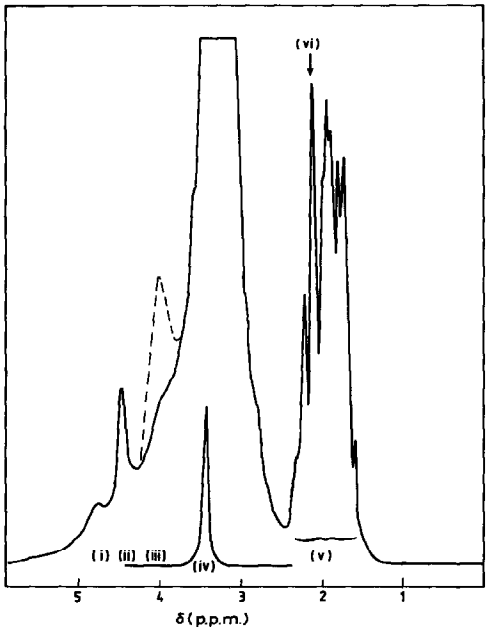

Fig. 2. ^{1}H-N.m.r. (HO) spectrum for 1.4M D-fructose at 0°: pH 6 (—), pH 7.2 (---); (i) HO-2 (*β-f*) at pH 6.0, (ii) HO-2 (*β-p*) at pH 6.0, (iii) HO-1,3,4,5 at pH 7.2 (this peak is not resolved at pH 6.0), (iv) H_2O, (v) C-H resonances, (vi) internal reference.

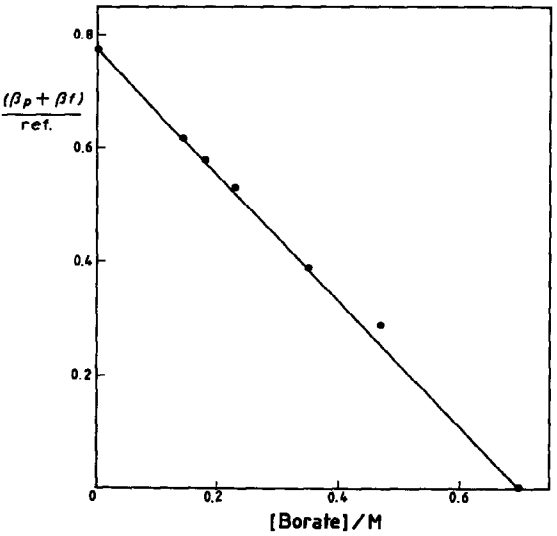

Fig. 3. ^{1}H-N.m.r. (HO) results at 0°, showing the change in the ratio (*β-p* + *β-f*)/ref. [*β-p*, *β-f* and ref. are the areas under peaks (i), (ii), and (vi), respectively, as shown in Fig. 2].

hydroxyl groups, the resonance of one has not shifted, one has complexed and the resonance is lost, one has a normal resonance shifted downfield (detected), and one resonance has been shifted upfield (hidden).

^{13}C-N.m.r. spectra of anomeric protons. — Gorin *et al.*[5] suggested that, by using ^{13}C-n.m.r. spectra, borate complexing could be detected by observing line-broadening (assigned to carbons directly attached to the oxygen ligands) and by observing the growth of new bands. It is not clear what circumstances govern the appearance of those alternative phenomena. The first criterion clearly failed in the present research since resonances due to C-1,4,5,6 were broadened but those for C-2,3 were unchanged in width. Hence, broadening cannot be taken as a measure of complexing by the relevant OH groups.

Our results confirm that certain new peaks are gained which are clearly due to the complexes. All the sugar ^{13}C resonances decreased in intensity on adding borate, with the concomitant growth of new bands. The assignments given in Table I and Fig. 4, which are not explicitly discussed, are taken as the most reasonable. In several instances, complex bands were too close to the parent bands for clear assignments to be made.

Most of the new peaks assigned to the complexes appear as multiplets (Fig. 4). By measuring ^{13}C-n.m.r. spectra of the same solution at two different frequencies, it was shown that these arise from species having signals with slightly different chemical shifts. This finding is interpreted in terms of the presence of at least two slightly different complexes. One possibility is that there is a significant contribution from the mixed complex β-*f* + β-*p* (borate) having ^{13}C resonances for the anomeric carbons close to, but not identical with, those for the non-mixed complexes.

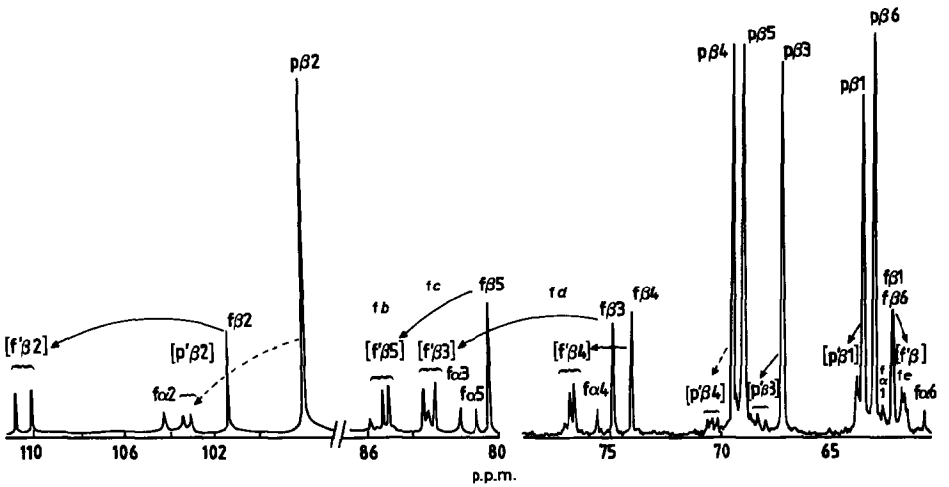

Fig. 4. ^{13}C-N.m.r. spectrum of 1.4M D-fructose in 0.2M borate at 0°. Peak assignments for D-fructose have been taken from published data[12]. The labels assigned to borate complexes are given in parentheses.

TABLE I

RESULTS TAKEN FROM FIG. 4, SHOWING THE AVERAGE PEAK SHIFTS (P.P.M.) OF ^{13}C PEAKS DUE TO COMPLEXING[a]

Furanose form	*Shifts*	*Pyranose form*	*Shifts*
fβ1→?	−0.5	pβ1→[p′β1]?	0.3
fβ2→[f′β2]	9.2	pβ2→[p′β2]	5.3
fβ3→[f′β3]	8.2	pβ3→[p′β3]?	1.3
fβ4→[f′β4]	2.8	pβ4→[p′β4]?	1.1
fβ5→[f′β5]	5.0	pβ5→?	?
fβ6→?	?	pβ6→?	?

[a]Assignments are discussed in the text. Ambiguities are indicated with a "?".

It is clear that the anomeric ^{13}C resonances are shifted strongly downfield (Fig. 4), especially for the furanose form. Since this forms the most stable complex, it seems probable that the magnitude of the shifts reflects the strength of the bonding. On this basis, it is suggested that peaks *fb* (Fig. 4) originate from C-5, and *fc* from C-3, with *fd* from C-4; hence, *fe* is from C-1. These assignments were chosen to minimise the shifts, and show that the C-2 (anomeric) shifts are about equal to the C-3 shift which is far greater than those of the remainder (Table I). This view strongly supports the concept that complexing involves HO-2,3.

For the pyranose form, which complexes less favourably, the shift of the signal for C-2 is considerably reduced and it was not possible to decide from the observed peaks which of the two alternative OH groups, *i.e.*, HO-1 or HO-2, was involved in complexation. That either can be used to form conformationally reasonable complexes is readily shown by molecular models.

When 0.5 equiv. of borate was added and the solution was left to equilibrate, almost all the free sugar molecules were complexed. At room temperature, the concentration of furanose complex was approximately three times that of pyranose complex. However, at 60°, the complex was almost entirely in the furanose form. These results confirm that this form is the most stable and that the interconversion rates are low.

Excess borate. — In the presence of an excess of borate, ^{13}C peaks assigned to the 2:1 complex gave way to further new peaks. Clearly, some of the remaining OH groups were being complexed by borate, but no attempt was made to study the resulting spectra because of their poor definition.

Thus, it is concluded that 2:1 complexes are favoured and that, as expected, the anomeric OH groups are always involved. Almost certainly, the other OH group is HO-3 which is sterically the most reasonable group to be involved.

EXPERIMENTAL

^1H-N.m.r. (OH) spectroscopy. — Spectra were recorded with a Jeol PS100

spectrometer between 0° and −5° (Comark thermocouple). Measurements were made rapidly after cooling, since certain solutions tended to phase-separate on long storage at these temperatures. The concentration of D-fructose was calculated by measuring the areas of the selected bands, the area of one of the C-H resonances being used as a reference.

^{13}C-N.m.r. spectroscopy. — Proton-decoupled spectra (internal Me_2SO, δ 40.6 relative to that of Me_4Si) were recorded at 300 or 400 MHz with Bruker AM300 and WH400 instruments.

pH Measurements. — The effective pH value of each solution was measured with a PHM83 Autocal pH meter; there are unique pH values at which the OH proton resonances for different OH groups have minimum widths[9]. These optimum pH values were 6.0 ±0.1 and 7.2 ±0.1 for the anomeric and non-anomeric OH protons, respectively, independent of the borate concentration. The pH was modified by the addition of aqueous NaOH or HCl.

Preparation of solutions. — D-Fructose and sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$) were of the highest grades available. D-Fructose was dried *in vacuo* at ~62° before use. Water was distilled prior to use. Weighed quantities of sodium tetraborate were added to a weighed quantity of D-fructose, and the mixtures were dissolved. Spectroscopic studies were undertaken after it had been ensured that reactions were complete, by storing the solutions at room temperature for several hours.

For ^{13}C-n.m.r. spectroscopy, the pH was adjusted to ~8. Minimum concentrations of D_2O (for locking) and Me_2SO were then added and the solution was diluted to the required concentration.

ACKNOWLEDGMENT

We thank Dr. P. J. Somers (University of Birmingham) for helpful advice.

REFERENCES

1 H. S. Isbell, J. F. Brewster, N. B. Holt, and H. L. Frush, *J. Res. Natl. Bur. Stand.*, 40 (1948) 129–149.
2 J. Boeseken, *Adv. Carbohydr. Chem.*, 4 (1949) 189–210.
3 J. Dale, *J. Chem. Soc.*, (1961) 922–930.
4 G. R. Kennedy and M. J. How, *Carbohydr. Res.*, 28 (1973) 13–19.
5 P. A. J. Gorin and M. Mazurek, *Carbohydr. Res.*, 27 (1973) 329–339.
6 M. C. R. Symons, J. A. Benbow, and J. M. Harvey, *Carbohydr. Res.*, 83 (1980) 9–20.
7 B. Schneider, F. W. Lichtenthaler, G. Steinle, and H. Schiweck, *Liebigs Ann. Chem.*, (1985) 2443–2453.
8 S. A. Barker, H. Pelmore, and P. J. Somers, *Enzyme Microb. Technol.*, 5 (1983) 121–124.
9 N. Ingri, *Acta Chem. Scand.*, 17 (1963) 581–589.
10 N. Ingri, *Sven. Kem. Tidskr.*, 74 (1963) 199–230.
11 H. Pelmore and P. J. Somers, *Enzyme Microb. Technol.*, 4 (1982) 390–394.
12 H. Bock and C. Pederson, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.