Structures of Borate–Aldohexose and Borate–Chetohexose Complexes in Aqueous Solution. A Thermodynamic Study

Roberto Aruga
Department of Analytical Chemistry, University of Turin, via Giuria 5, 10125 Turin, Italy

Heats of complex formation of the borate ion with the carbohydrates D-galactose, D-glucose, D-fructose, D-mannose, and L-sorbose have been determined by direct calorimetry. By means of the equilibrium constants the corresponding Gibbs functions and entropies were also obtained. The present data refer to the aqueous medium, at T = 25 °C and I = 0.1 mol dm^{-3}. Examination of the results indicates a leading role, in several cases, of solute–solvent external factors (*e.g.* desolvations of the borate ion) in determining the different stabilities of the complexes. Amongst various factors for the ligands, some appear of minor importance, while the contribution of others (such as the strength of the borate—hexose C–O–B bonds) is fairly constant throughout one series. The present data seem also to confirm the presence of the furanose cyclic form of the carbohydrate in the complex.

The importance of the reaction of boric acid with polyhydroxy compounds in analytical chemistry and in the investigation of molecular structures is well known.[1] Such reactions could have a remarkable interest also in phytobiology, as the absorption of borate by many plants could take place through polyhydroxy compounds such as polysaccharides.[2,3]

At first, borate–carbohydrate complexes in solution were mainly investigated by means of the determination of stability constants, even at different temperatures.[4–10] It must be noted, in general, that these quantities are merely conditional constants. In fact, when they are defined, the expression of the concentration (or activity) of uncomplexed carbohydrate is the sum of the concentrations of various forms (*i.e.* α,β, pyranosic, furanosic, *etc.*) present in the solution, one only of which presumably reacts with borate (see below). Then the magnitude of the constants is dependent on the experimental conditions chosen. In other words these quantities are useful for calculating the actual concentration of the complex under particular conditions, but they may not lead to reliable and general conclusions about the stability and structural features of the complex.

Among spectral methods, n.m.r. has recently given some information on the borate esters with D-fructose, D-glucose, and other polyhydroxy compounds.[11]

As calorimetric values of enthalpy and entropy are not available in the literature for these associations,[12] it has been thought of interest, in the present work, to determine the above quantities by direct calorimetry for the reaction of borate with D-galactose, D-glucose, D-fructose, D-mannose, and L-sorbose in aqueous solution.

Experimental

Reagents and Solutions.—Analytical-grade reagents were always used: boric acid, C. Erba RPE-ACS 99.8%; D-galactose, D-glucose, D-fructose, D-mannose, and L-sorbose, Fluka puriss. Ionic strength and pH were adjusted to the desired values by sodium nitrate, sodium hydroxide, and nitric acid (C. Erba RPE). To avoid any influence by mutarotation, the carbohydrate solutions were used at least 24 h after preparation.

Equipment.—Calorimetric measurements were made at 25 °C with an LKB 8700-2 Precision Calorimetry System (isoperibol, incremental-titration type) and an LKB 8726-1 100-cm^3 titration vessel. Fuller details on the instrument and the calibration have been reported.[13] The calorimeter was equipped with a Radiometer ABU 12b autoburette for the addition of titrant. pH Measurements were carried out with a Metrohm 605 potentiometer. The calorimetric experiments were performed in a room kept at a temperature constant to within ±0.3 °C (Branca Idealair 'Zero' air-conditioning system).

Procedure.—Three or four series of measurements, with different concentrations of reagents, were carried out for each borate–hexose system. Each series was performed in the following manner. Successive amounts of sodium borate solution (2.505 ± 0.002 cm^3) were added to a hexose solution (88.00 cm^3) in the calorimetric vessel, and the heat for each addition measured. The hexose solution was previously brought to pH 5.2 ± 0.2. Sodium borate solutions were obtained from boric acid solutions brought to pH ≥ 11.6 by NaOH. The pH values and the borate–hexose concentration ratios were similar to those used in the determination of the corresponding stability constants.[5,7] In this way complex species of the type $[B_2L]^{2-}$ are absent (B^- = borate ion; L = carbohydrate). At the same time the above constants (which are conditional constants, see Introduction) may be used for a reliable evaluation of the amount of the complex species formed under the present conditions. The ionic strength of all solutions was adjusted to 0.1 mol dm^{-3} by $NaNO_3$. The corresponding heat of dilution was measured by adding the same amounts of the sodium borate solution to 0.1 mol dm^{-3} $NaNO_3$ (88.0 cm^3) (pH ≥ 11.6), without any carbohydrate.

The following results were obtained under these conditions. (*a*) A negligible heat of neutralization between H^+ and OH^-. (*b*) A negligible heat of reaction between the $[B(OH)_4]^-$ ion and the proton. By assuming a pK_a value of 9.1 for boric acid at 25 °C and I = 0.1 mol dm^{-3} (ref. 7) and ΔH° = 13.8 kJ mol^{-1} for its dissociation,[14] a heat of smaller than 0.02 J was obtained for this reaction. (*c*) A negligible contribution to the measured heats from dissociation of polyborate species: since the borate concentration was lower than 0.025 mol dm^{-3} at the end of the mixing process (Table 1), it must have reacted in the monomeric form[15] and, moreover, the heat of dissociation of any polyborates which may be present in the initial solution is counterbalanced by an equal heat effect during the dilution experiment, so that it is eliminated in the calculation of the corrected heat (see below). The heat for the partial deprotonation of the hexoses was also calculated. It was found to be not negligible in some cases. The experimental heats were then corrected for this process (see treatment of the experimental data). The pH values in the cell were measured potentiometrically, by means of a titration performed in the same way as the calorimetric one. Finally, it must be noted that

Table 1. Experimental data for the mixing of aqueous solutions of sodium borate and hexoses (L) at 25 °C*

L	$10c_B$/mol dm^{-3}	$10c_L$/mol dm^{-3}	pH_f	$10^3[BL^-]$/ mol dm^{-3}	$10^3[BL_2^-]$/ mol dm^{-3}	10[L]/ mol dm^{-3}	ΣQ_c/J	ΣQ_{cc}/J
D-Galactose	1.30	4.00	9.03	2.25	1.31	3.84	2.43	2.22
			9.23	4.44	2.48	3.69	5.06	4.74
			9.35	6.58	3.53	3.55	8.12	7.70
			9.44	8.66	4.47	3.41	11.2	10.8
D-Glucose	1.00	2.99	9.57	1.46	1.28	2.87	3.25	2.54
			9.90	2.89	2.44	2.75	6.48	5.00
			10.08	4.30	3.49	2.64	9.88	7.67
			10.20	5.68	4.43	2.54	13.2	10.4
D-Fructose	1.30	0.90	8.80	1.25	2.34	0.81	8.05	8.00
			9.20	2.60	4.39	0.74	15.4	15.3
			9.43	4.50	6.16	0.66	22.5	22.3
			9.57	5.60	7.67	0.60	28.8	28.6
D-Mannose	1.30	2.00	9.10		3.59	1.87	2.63	2.26
			9.33		6.99	1.75	5.02	4.41
			9.47		10.2	1.64	7.48	6.68
			9.56		13.3	1.53	9.78	8.84
L-Sorbose	1.30	2.00	7.90		3.60	1.87	8.39	8.38
			8.70		7.00	1.75	16.6	16.5
			8.94		10.2	1.64	24.6	24.5
			9.06		13.3	1.53	32.8	32.6

* Sodium borate was added to 88.00 cm^3 of L in the calorimetric cell in four successive amounts. Cumulative volumes added: 2.505, 5.010, 7.515, and 10.020 cm^3.

Table 2. Molar thermodynamic quantities for complex formation reactions of the borate ion, $[B(OH)_4]^-$, with hexoses in aqueous solution at 25 °C and I = 0.1 mol dm^{-3}

Hexose	j[a]	log K_j[b]	$-\Delta G_j^\circ$/ kJ mol^{-1}	ΔH_j°/ kJ mol^{-1}	ΔS_j°/ J K^{-1} mol^{-1}
D-Galactose	1	2.21 ± 0.03[c]	12.6 ± 0.2	−24.7 ± 1.7	−42 ± 4
	2	0.18 ± 0.17	1.0 ± 0.8	48.5 ± 4	167 ± 12
	1 + 2	2.39 ± 0.17	13.6 ± 0.8	24 ± 4	125 ± 12
D-Glucose	1	2.27 ± 0.05	12.9 ± 0.3	−17 ± 1	−12.5 ± 4
	2	0.49 ± 0.05	2.8 ± 0.3	15 ± 3	58 ± 8
	1 + 2	2.76 ± 0.07	15.7 ± 0.4	−2 ± 3	46 ± 9
D-Fructose	1	3.58 ± 0.05	20.4 ± 0.3	−3 ± 1	59 + 4
	2	1.36 ± 0.05	7.7 ± 0.3	−33 + 2	−84 ± 4
	1 + 2	4.94 ± 0.07	28.1 ± 0.4	−36 ± 2	−25 ± 6
D-Mannose	1 + 2	4.42 ± 0.05	25.2 ± 0.3	−6.81 ± 0.02	61.5 ± 0.8
L-Sorbose	1 + 2	5.80 ± 0.05	33.1 ± 0.3	−25.15 ± 0.08	26.8 ± 0.8

[a] j = 1 or 2 for stepwise reactions: $[BL_{j-1}]^- + L \longrightarrow [BL_j]^-$ (B^- = borate, L = hexose); j = 1 + 2 for overall reactions: $B^- + 2L \longrightarrow [BL_2]^-$. [b] See refs. 5 and 7. [c] The uncertainty given in each case is the estimated standard deviation.

the potentiometric and calorimetric experiments showed that the achievement of the equilibrium in the cell, after each addition of titrant, was rapid enough for carrying out correct calorimetric measurements with the LKB 8700–2 system.

Treatment of the Experimental Data.—The heat of deprotonation of the hexoses was calculated by using the corresponding pK and ΔH° values of acid dissociation reported previously.[16] Some of the experimental data are collected, as an example, in Table 1, where c_B is the initial total concentration of borate in the titrant solution, c_L is the initial concentration of hexose in the calorimetric vessel, pH_f, $[BL^-]$, $[BL_2^-]$, and [L] are the pH and concentrations of the various species after each addition of titrant, and where ΣQ_c and ΣQ_{cc} are the cumulative heats, corrected for dilution and for dilution and deprotonation respectively.

Molar enthalpies of association were determined from the experimental heats (ΣQ_{cc}) and the concentrations of the complex species: the latter were calculated from values of the corresponding stability constants determined potentiometrically[5,7] under the conditions of temperature and ionic strength used in the present study. No contraction in volume was found (in the limits of the instrumentation used) on mixing the reagents. The ΔH° values and the corresponding standard deviations were calculated by means of the numerical method of minimization of the error square sum for each measurement.[17] In order to check the enthalpy values, the experimental heats were recalculated by using the ΔH° values obtained. Entropies were calculated by means of the equation: $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$. The enthalpy values calculated by taking into account only some additions of titrant were equal to the values obtained from the entire titration process. This shows that the conditions of increasing pH in the calorimetric cell in the course of the titration (Table 1) do not alter the hexose molecule in the time required for a measurement.

Results and Discussion

The molar thermodynamic quantities of complex formation are collected in Table 2. No stepwise quantities are listed for D-mannose and L-sorbose, as only $[BL_2]^-$ was found for these ligands in the cited works.[5,7]

No previous calorimetric data are available on the present equilibria. On the other hand comparisons of the present results

with those obtained from equilibrium constants at various temperatures do not seem reliable. The latter, in fact, may be so inconsistent that sometimes they differ even in sign [the following entropy values, for instance, have been obtained for the successive steps of the borate–D-glucose complex formation: $\Delta S_1 = 20$, $\Delta S_2 = -34$ (ref. 9); $\Delta S_1 = -27$ and $\Delta S_2 = 31$ J K^{-1} mol^{-1} (ref. 10)].

Examination of the data in Table 2 shows that the factors which favour the formation of the present complexes are not attributable in a simple way to enthalpy or entropy alone. These quantities appear to be quite different, both for aldoses in comparison with chetoses and for the two steps of reaction. For D-galactose and D-glucose, for instance, the first step is favoured by enthalpy and opposed by entropy, while an opposite behaviour is shown in the second step. In the case of D-fructose the general trend appears reversed in comparison with that of the two aldoses.

The following factors should be taken into consideration for a correct examination of the problem of complex stability in the present case. (*a*) The stability of the C–O–B ester bonds, which are the fundamental link between borate and hexose: –C–OH + HO–B– ⟶ –C–O–B– + H_2O. (*b*) The presence of hydrogen bonds. (*c*) The difference in stability between the complexed and uncomplexed form of hexose. This contribution may assume a leading role in certain ring structures. The *myo*-inositol–borate complex, for instance, is very weak, in spite of the presence of three C–O–B bonds. This fact is due to the high energy content of the 'chair' structure of *myo*-inositol in the complex compared with that of the usual chair.[18] (*d*) Solute–solvent interactions. (*e*) Hexose–hexose interactions; in the case of 1:2 complex species, mainly of steric nature.

In order to evaluate the importance of factor (*a*) it is necessary to ascertain in which form the hexose molecule takes part in the complex formation. It was found from n.m.r. data[11] that D-fructose and, probably, D-glucose are present in the 1:2 complex with borate almost entirely in the furanose cyclic structure (see Figure).

In the course of the present calorimetric experiments with D-galactose and L-sorbose, a small endothermic effect, slightly greater than the experimental error, was repeatedly observed at the end of the exothermic main process. Taking into account that the present hexoses are prevailingly present in solution in the pyranose conformation[19,20] and that some previous calorimetric measures indicate that the pyranose ⟶ furanose isomerization is endothermic,[20] the above mentioned endothermic effect may be considered as confirmation of the n.m.r. results. The fact that no additional thermal effect was observed for some of the hexoses may be explained by considering that the pyranose–furanose isomerization is rather fast under the present conditions of pH.[19] Consequently the heat of isomerization could be entirely superimposed to the heat of complex formation in these cases. Possible exchanges between α and β forms during complex formation should give negligible thermal effects, as the corresponding molar enthalpies were found to be very low.[20]

The furanose form of carbohydrate in the present complexes can be justified by examining the possible C–O–B bond pairs formed by the two cyclic isomers of the hexoses. Hexopyranoses could give two C–O–B bonds through two adjacent OH groups of the ring. Owing to the different orientation of these groups (*i.e.* axial and equatorial) and the semi-rigid ring structure, these bonds would be fairly unstable.[7] Two bonds with 1,3 OH groups of the ring in 'parallel' position would also be possible, but, in this case too, they are not very stable.[18] Finally, two bonds with the OH groups on the carbon atoms in positions 1,2

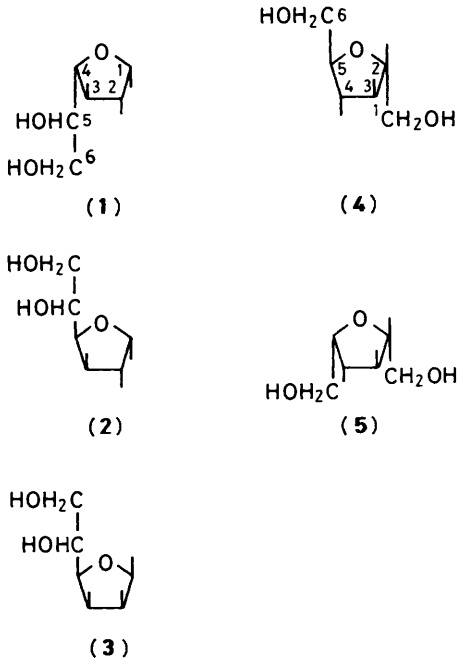

Figure. Structural schemes of aldohexoses (left) and chetohexoses (right) in the furanose form: α-D-galactofuranose (**1**), α-D-glucofuranose (**2**), β-D-mannofuranose (**3**), β-D-fructofuranose (**4**), and α-L-sorbofuranose (**5**). The numbering of the carbon atoms is exemplified in (**1**) for aldoses, in (**4**) for chetoses

are possible for borate with chetopyranoses. Such bonds are strong, owing to the ease of orientation of the hydroxy groups of hexose. They are quite similar to the usual pair of bonds in the borate esters of 1,2-glycols. In the case of the furanose ring a strong binding (quite similar to the preceding one) could be formed through the two OH groups of the open chain in positions 5,6. This is possible for aldoses only (Figure). Moreover the difference in orientation (equatorial, axial) of two adjacent OH groups in *cis* position is much smaller in the furanose ring ('envelope' conformation) than in the chair conformation of the pyranose ring. The OH groups of the former ring are easily oriented as in the case of the 1,2-diol open chain (see ref. 21 and Corey–Pauling–Koltun, CPK, models). At the same time the corresponding pair of bonds with borate should lead to an even more stable structure in the case of the furanose ring than of 1,2-diol, for entropy reasons. Owing to the quasi-rigid ring structure, in fact, the formation of the bonds takes place, in this case, with a smaller loss of conformational entropy than for the open chain. It can then be concluded, from the preceding experimental and structural observations, that the hexoses are present in the borate complexes in the cyclic furanose form. As concerns the α or β configuration of hexofuranose, that which possesses a pair of OH groups in *cis* position on carbon atoms 1,2 for aldoses (or 2,3 for chetoses) should react with borate. If the structure proposed here is the actual one, then factor (*a*) above has a fairly constant influence on the stability of the various complexes.

Possible structures of each borate–hexose ester have been investigated by means of CPK models also. As regards the 1:1 complex of D-galactose (in the form of α-D-galactofuranose), besides two C–O–B bonds on the 1,2 carbon atoms, hydrogen bonds can be present between the OH groups on the 5,6 carbon atoms of the side chain and one OH group of borate (Figure). The consequent loss of conformational freedom by the side chain, together with the fact that no charge neutralization takes place in these reactions, could account for the negative ΔS_1°

value (-42 ± 4 J K^{-1} mol^{-1}). Taking into account the compact arrangement of borate and sugar in the 1:1 species, a partial detachment and a regain of conformational freedom of the first ligand should be caused by the formation of the 1:2 borate–D-galactose species. The more positive enthalpy and entropy for the second than for the first step can be justified, at least partially, in this way. It must be noted, in any case, that the strong positive ΔS_2° for D-galactose does not seem to be explained through this factor alone.

The difference of α-D-glucofuranose compared with α-D-galactofuranose lies in the position of the 5,6 side chain, which is now opposite to borate in the complex species. As no immobilization in the first step as well as no detachment in the second step is possible for the side chain of the hexose in this case, the less negative ΔS_1° and the less positive ΔS_2° for D-glucose than for D-galactose can be explained in this manner. The corresponding enthalpies too are in qualitative accordance with this explanation.

The main structural features of β-D-fructofuranose in comparison with aldofuranoses lie in the two CH_2OH groups in positions 2 and 5. From CPK models, the hydroxymethyl groups of D-fructofuranose give two hydrogen bonds with the two unbound OH groups of borate. Moreover, in this case the structure of the ligand being more rigid than in the case of D-galactofuranose, hydrogen bonds are formed with a smaller loss of conformational freedom. β-D-Fructofuranose can then be considered as a structure well enveloping the borate ion and causing, presumably, strong desolvation phenomena of the latter. The clearly positive ΔS_1° for D-fructose, together with less exothermic ΔH_1° values than for the two aldoses are in agreement with this possibility. No steric factors, in the second step, should oppose the formation of bonds as strong as in the first step, while desolvation processes of borate should be nearly absent now. The most favourable ΔH_2° together with the most unfavourable ΔS_2° among the stepwise values in Table 2 agree with this possibility for D-fructose.

β-D-Mannofuranose has a structure similar to that of α-D-galactofuranose. Moreover, the 1,2,3 OH groups are all in *cis* position in the former hexose. Such a structure, as can be inferred from CPK models, leads to a compact borate–hexose arrangement by means of hydrogen bonds, and to possible desolvation processes almost as strong as for D-fructose. The similar behaviour of D-mannose compared with D-galactose and D-fructose is well reflected by the overall enthalpy and entropy values. In fact, as can be seen in Table 2, the quantities for D-mannose are intermediate between those of the other two hexoses.

The two CH_2OH groups are oriented differently in α-L-sorbofuranose than in β-D-fructofuranose, so that different solute–solvent interactions should be present for the two chetoses in the two steps of complexation. In any case, the availability for L-sorbose of overall values only does not allow one to draw conclusions on this point.

In conclusion, the present calorimetric data do not confirm previous explanations of different stabilities only based on internal factors. The present results, in particular, do not confirm the conclusions of a previous study,[7] according to which the higher chelating ability of chetoses in comparison with aldoses should be caused by ester bonds with OH groups outside the pyranose ring (positions 1 and 2) for the former, and with OH groups of the pyranose ring for the latter. It can be concluded, on the other hand, that in several cases solute–solvent interactions are of great importance, among the above mentioned factors, in determining differences in complex stability. Desolvation processes of the borate ion, in particular, seem to play a leading role.

Among the other factors cited, the contribution of some is fairly constant throughout the series of ligands such as C–O–B bonds, some (such as hydrogen bonds or hexose–hexose interactions in the 1:2 species) seem to have a minor influence. As regards the change from the pyranose to the furanose structure of hexoses, the minor heat attributable to this process in comparison with the total heat should lead to the conclusion that this factor too is of minor importance.

References

1 J. Böeseken, *Recl. Trav. Chim. Pays-Bas*, 1942, **61**, 82.
2 M. E. Winfield, *Aust. J. Exp. Biol. Med. Sci.*, 1945, **23**, 111.
3 W. J. Evans, V. L. Frampton, and A. D. French, *J. Phys. Chem.*, 1977, **81**, 1810.
4 G. L. Roy, A. L. Laferriere, and J. O. Edwards, *J. Inorg. Nucl. Chem.*, 1957, **4**, 106.
5 P. J. Antikainen, *Suom. Kemistil. B*, 1958, **31**, 255.
6 J. P. Lorand and J. O. Edwards, *Org. Chem.*, 1959, **24**, 769.
7 P. J. Antikainen and K. Tevanen, *Suom. Kemistil. B*, 1959, **32**, 214.
8 E. W. Malcom, J. W. Green, and H. A. Swenson, *J. Chem. Soc.*, 1964, 4669.
9 P. J. Antikainen and I. P. Pitkänen, *Suom. Kemistil. B*, 1968, **41**, 65.
10 J. M. Conner, *J. Inorg. Nucl. Chem.*, 1970, **32**, 3545.
11 M. Makkee, A. P. G. Kieboom, and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 1985, **104**, 230.
12 J. J. Christensen and R. M. Izatt, 'Handbook of Metal Ligand Heats,' Marcel Dekker, New York, 1983.
13 R. Aruga, *Aust. J. Chem.*, 1981, **54**, 501.
14 M. J. L. Tillotson and L. A. K. Staveley, *J. Chem. Soc.*, 1958, 3613.
15 T. Paàl, *Acta Chim. Acad. Sci. Hung.*, 1976, **91**, 393.
16 J. J. Christensen, J. H. Rytting, and R. M. Izatt, *J. Chem. Soc. B*, 1970, 1646.
17 L. G. Sillén, *Acta Chem. Scand.*, 1962, **16**, 159.
18 S. J. Angyal and D. J. McHugh, *J. Chem. Soc.*, 1957, 1423.
19 W. Pigman and J. Sowden, in 'The Carbohydrates,' ed. W. Pigman, Academic Press, New York, 1957.
20 S. Ono and K. Takahashi, in 'Biochemical Microcalorimetry,' ed. H. D. Brown, Academic Press, New York, 1969.
21 L. Hough and A. C. Richardson, in 'Rodd's Chemistry of Carbon Compounds,' ed. S. Coffey, Elsevier, Amsterdam, 1967, vol. I^F, p. 113.

Received 23rd *October* 1987; *Paper* 7/1896