

THE REACTION OF BORATE AND SUGARS.

III. THE FREEZING POINT LOWERING OF SUGARS IN BORAX SOLUTIONS.*

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The interpretation of our data on the optical activities of sugars in borax solution and the effects attending the acidification of these solutions (Levy and Doisy, 1929) required a knowledge of the amount of sugar combined in the solution. Three possible methods may be outlined. The most direct would be to prepare in a pure state the compounds of the various sugars and borate and establish by analysis their composition, and from some property, perhaps the optical activity, establish a method for the determination of the reaction product in our equilibrated solutions. However, we have not been able to isolate such compounds.

A second method depending on the determination of the equilibrium in the solution between boric acid and its salts, as determined by the pH of the solutions, has been outlined in the preceding paper. This method really determines the amount of borate ion entering into complex formation and not the amount of sugar. Only by assuming the composition of the compound could this be related to the amount of sugar combined. Since it was not possible to isolate the compounds, it would be necessary here to reason by analogy. Hermans (1925) has stated that the complex formed with boric acid and hydroxy compounds contains 2 molecules of hydroxy compound per molecule of boric acid if the

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conductivity of boric acid is increased, and only 1 molecule if it is not increased. The distinction between the two classes of compounds on the basis of conductivity seems to call for some comment. Theoretically, a complex formed between 2 molecules of hydroxy compound and 1 molecule of boric acid should be a stronger acid than the boric acid, as has been shown by Hermans. It is not necessarily true, however, that a compound of the 1:1 type will be weaker than boric acid or of the same strength. The dissociation of the acid hydrogen would be influenced by the nature of the hydroxy component entering the complex, and the possibility that the complex could be dissociated to a greater extent than boric acid and so cause an increase in conductivity is not excluded. It appears then that the second method of study is uncertain with regard to the amount of sugar combined because the composition of the complex is unknown.

The third possible method is the study of one of the colligative properties of the solution and the most convenient of these is the freezing point. If no compound formed between borax and the sugar, the freezing point lowering of the mixed solution should be the sum of the freezing point lowerings due to each component. If a complex is formed, the freezing point lowering will be less than the sum, and the difference between the sum and the observed lowering divided by the molecular freezing point lowering should give the concentration of combined sugar; comparison of this value with the amount of borate combined as determined by the second method should establish the composition of the compound.

For the present purpose, a borax solution may be considered as containing equimolecular amounts of NaBO_2 and HBO_2 (Kahlenberg and Schreiner, 1896). The freezing point lowering of the borax solution is accounted for by the partial lowerings caused by Na^+ , BO_2^- , NaBO_2 , and HBO_2 . The dissociation of HBO_2 can be disregarded, since it is a weak acid in the presence of its salt. Complex formation with HBO_2 is negligible (as a first approximation) as shown by the lack of a measureable change of the optical activities of sugars in its presence. The complex with which we are dealing exists in the solution in the form of its salt and the freezing point lowering of a solution containing borax and sugar may be attributed to the sum of the partial lowerings due to Na^+ , BO_2^- , NaBO_2 , HBO_2 , compound ion, undissociated salt of the

compound, and free sugar. Part of the borate ion and sodium borate in the borax solutions has been replaced by the compound ion and undissociated sugar-borate compound. There is no reason for assuming that this compound will be other than monovalent; consequently, neglecting the difference in degree of dissociation of the two salts, we may assume that the partial freezing point lowering due to the NaBO_2 entering into complex formation will be replaced by the partial lowering due to the complex and its salt. If we subtract the lowering of borax solution from the observed lowering, the difference will be due to the free sugar, and this divided by the value of the molecular freezing point constant will give the value of the free sugar concentration. The difference between the amount of sugar added and the amount of free sugar is the amount of sugar entering the complex.

The second method can be used to show the ratio of total borate to boric acid and if we neglect any compound formation with the acid, the difference between the original and final $[\text{BO}_2^-]$ will be the amount of borate entering into compound formation. A comparison of the results of the two methods will give an insight into the composition of the compound formed.

The difference in the degree of dissociation of the sodium salt of the compound and of sodium borate will disturb the results by the freezing point method. If the complex salt is ionized to a greater extent than NaBO_2 , then the amount of combination will be greater than that actually calculated from the freezing point data, and if the complex is dissociated to a lesser extent, the complex formation will be less than calculated. This variation should not be greater than 20 per cent, however, since the degrees of dissociation of most salts at the concentrations we have used vary over a range of 0.60 to 0.95.

With the limitations in mind, we may use the data of Kahlenberg and Schreiner (1896) on the freezing points of mannitol-borax solutions to illustrate the method of calculation. They found that Δ_B for 0.1 M borax solution was 0.720° and for a solution containing 0.4 M mannitol in addition to 0.1 M borax $\Delta_S = 1.120^\circ$. The difference between the two ($\Delta_S - \Delta_B$) is 0.400° . This divided by 1.86° (the molecular freezing point lowering for water) gives 0.215 mols as the amount of free mannitol. The amount combined is then $0.4 - 0.215 = 0.185$ mols. Similar calculations

for other concentrations of mannitol from data given by Kahlenberg and Schreiner are shown in Table I.

It is evident from Table I that 0.2 M mannitol enters into compound formation with 0.1 M borax. The solutions are neutral or slightly acid (our observation) consequently no free borate remained in the solution, and since it was originally 0.2 M in borate, the complex in the solution was formed between 1 molecule of NaBO_2 and 1 molecule of mannitol, corresponding to the monomannito-monoboric acid isolated by Fox and Guage (1911), the existence of which Hermans doubted because he was unable to prepare it and because of theoretical considerations.

TABLE I.
Amount of Mannitol Involved in Complex Formation with Borate.

Data of Kahlenberg and Schreiner.

Borax, μ	0.1	0.1	0.1	0.1	0.1
Mannitol, μ	0.2	0.4	0.6	0.8	
Δ , degrees.....	0.792	1.120	1.449	1.845	0.720
$\Delta_S - \Delta_B$, degrees.....	0.072	0.400	0.729	1.125	
Free mannitol, μ	0.039	0.215	0.392	0.605	
Combined mannitol, μ	0.161	0.185	0.208	0.195	

Methods.

Freezing points were determined by the usual arrangement except that in order to conserve certain of the materials, a micro-Beckmann thermometer and apparatus suitable for the use of 10 cc. of solution were used. Δ_B of 0.05 M borax solutions (0.2 M $\text{H}_3\text{BO}_3 + 0.1$ M NaOH) was determined and the freezing point lowering of the sugar alone in each case as a check. The sugar solutions were prepared by adding 10 cc. of water or 0.05 M borax to the weighed quantity of sugar so that the concentrations were approximately weight molar and disturbances due to displacement of water by the solute in the volume system were avoided.

The sugars and other materials used were described in the preceding paper (Levy and Doisy, 1929).

EXPERIMENTAL AND DISCUSSION.

The results are shown in Table II.

The concentration of combined sugar, which is the significant value in Table II, is shown in the last column. Every solution

TABLE II.

Compound Formation in Borax-Sugar Solutions from Freezing Point Data. $\Delta_{\text{borax}} = 0.426^\circ$. Each solution contained 0.05 M borax.

Sugar.	Concentration.	Δ_s	$\Delta_s - 0.426$	Free sugar.	Combined sugar.
	<i>M</i>	<i>degrees</i>	<i>degrees</i>	<i>M</i>	<i>M</i>
<i>d</i> -Glucose.	0.5	1.151	0.725	0.390	0.110
	0.4	0.981	0.555	0.298	0.102
	0.25	0.696	0.270	0.145	0.105
	0.2	0.607	0.181	0.097	0.103
<i>d</i> -Mannose.	0.5	1.171	0.745	0.400	0.100
	0.25	0.745	0.399	0.172	0.078
<i>d</i> -Galactose.	0.5	1.110	0.684	0.368	0.132
	0.25	0.650	0.224	0.121	0.129
<i>d</i> -Fructose.	0.5	1.075	0.649	0.349	0.151
	0.25	0.605	0.179	0.096	0.154
<i>l</i> -Rhamnose.	0.5	1.265	0.839	0.453	0.047
	0.25	0.797	0.371	0.199	0.051
<i>l</i> -Xylose.	0.5	1.103	0.704	0.378	0.122
	0.25	0.680	0.254	0.139	0.111
<i>l</i> -Arabinose.	0.5	1.185	0.759	0.408	0.092
	0.25	0.695	0.269	0.145	0.105
Maltose.	0.5	1.179	0.753	0.405	0.095
	0.25	0.735	0.309	0.166	0.084
Lactose.	0.5	1.264	0.838	0.452	0.048
	0.25	0.777	0.351	0.189	0.061
2,3,4,6-Tetramethylglucose.*	0.5	1.525	1.099	0.592	0.000
	0.25	0.951	0.525	0.284	0.000
Methylglucoside.	0.5	1.362	0.936	0.503	0.000
	0.25	0.864	0.438	0.236	0.014

* This derivative gave an abnormally high Δ in water, possibly because of impurities. This explains the high values of free sugar.

which showed a pH value less than 8.0 gave a value of combined sugar sufficiently close to 0.1 M to justify the conclusion that the compounds which are formed are made up of 1 molecule of sugar and 1 molecule of NaBO_2 . A pH value of 8 or lower indicates that practically all of the NaBO_2 originally present in the borax has been used up in complex formation. It is interesting to note that halving the amount of sugar added had comparatively little, if any, effect on the extent of complex formation. However, in every case the solution to which the greater amount of sugar had been added was the more acid as determined by the behavior of indicators. The increased acidity probably indicated complex formation with boric acid itself. In those cases where the amount of combined sugar was considerably higher than 0.1 M, the solutions showed distinctly lower pH values. Since the solution of fructose (0.5 M) and borax gives a colorimetric pH of 5.5, there can be little doubt that part of the combined sugar (0.15 M) reacts with boric acid, the difference being exaggerated perhaps by experimental error and differences in degrees of dissociation of the complex salt and NaBO_2 . The other two sugars which show high values for combined sugar (galactose, 0.13 and xylose, 0.12) give pH values of 6.5 in solutions containing 0.5 M sugar and 0.05 M borate. It should be noted here that none of the sugars tested by Levy and Doisy (1929) showed any change in optical activity in boric acid solution. However, evidence regarding the possible combination of boric acid with those sugars (fructose, galactose, and xylose) which show high values for combined sugar in Table II might be obtained by the use of Barfoed's copper reagent or by the cryoscopic method used in this paper. Those sugars (glucose, mannose, arabinose) which give values for combined sugar approximating 0.1 M, have pH values very close to 7. Rhamnose, maltose, and lactose solutions have pH values above 8 and in each case the amount of combined sugar is less than 0.1 M, significantly so with lactose and rhamnose. It may be noted that maltose and lactose showed less interference by borate in the oxidation of the aldehyde group by alkaline iodine than the other sugars and that this was interpreted to indicate a lesser extent of compound formation (Levy and Doisy, 1928). We believe that the data of Table II show quite definitely that compounds of sugars and borate con-

taining 2 molecules of sugar per molecule of borate do not exist to any great extent in our solutions.

In no case was the freezing point of the solution observed to change with time, indicating that the amount of compound formed did not change and that the mutarotation of various sugars in borax solutions (Levy and Doisy, 1929) was merely due to the excess sugar. The two sugar derivatives, 2, 3, 4, 6-tetramethylglucose and methylglucoside, show no evidence of combination with borax by the freezing point method, just as the optical method gives no evidence of combination (Levy and Doisy, 1929). This is additional evidence to show that complex formation in sugars occurs between the hydroxyl group of the aldehyde carbon atom and the hydroxyl group of carbon atom 2.

CONCLUSIONS.

1. Freezing point measurements in borax solutions of sugars show that compound formation occurs.
2. The compound which is formed is probably the product of the reaction of 1 molecule of sugar and 1 of NaBO_2 .
3. When the aldehydic hydroxyl group is blocked, as in methylglucoside, or when the hydroxyl group of carbon atom 2 is blocked, as in tetramethylglucose, complex formation does not occur.

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