

## THE REACTION OF BORATE AND SUGARS.

### II. THE OPTICAL ACTIVITY OF SUGARS IN BORAX SOLUTION AND THE CONFIGURATION OF MUTAROTATORY ISOMERS.\*

BY MILTON LEVY AND EDWARD A. DOISY.

(From the Laboratories of Biological Chemistry, St. Louis University School of Medicine, St. Louis.)

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During our study of the effect of borate on the oxidation of sugars (Levy and Doisy, 1928), our attention was attracted to the investigations of the effect of borax on the optical activity of glucose and fructose apparently first reported by Rimbach and Weber (1905). They observed that the optical activities of these sugars were less (*i.e.* nearer  $0^\circ$ ) in borax solutions than in distilled water but that the values of the optical activities were normal in boric acid solutions. They advanced the hypothesis that their observations could be explained adequately by the known effect of alkali upon carbohydrates and by formation of a compound between the borax and sugar.

Murchhauser (1923) observed the decreased rotatory power of glucose in the presence of borax and stated that this decrease was a specific effect of borax and indicated that probably the decrease had nothing to do with the alkalinity. He was impressed by the constancy of the final rotation at a much higher value than would be expected even with weakly alkaline solutions. This exceptional behavior had also been observed by Rimbach and Weber (1905) but the latter believed that this merely showed that the solution had become neutral through the formation of saccharinic acids

\* This paper and the previous one of this series (Levy and Doisy, 1928) are based on data taken from the Dissertation submitted by Milton Levy to the Graduate School of St. Louis University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

from the sugar. Michaelis and Rona (1912) have also observed the decreased rotation of glucose in borax solution.

Glucose (Boeseken, 1913), galactose and fructose (Boeseken, Kerstjens, and Klamer, 1916), xylose, arabinose, rhamnose, and mannose (Boeseken and Couvert, 1921) increase the conductivity of boric acid, and the  $\alpha$ - and  $\beta$ -isomers of each sugar are effective to different degrees. A consideration of the type of compound which Boeseken's systematic investigations have shown to be effective in increasing the conductivity of boric acid has led this author to assign to the  $\alpha$  and  $\beta$  forms of each sugar a definite configuration for the end aldehydic carbon atom.

The present experimental work began with observations made during the course of work on the effect of borate on the oxidation of sugars (Levy and Doisy, 1928) when it was shown that the effect of borax on the optical activity of glucose was reversible in the sense that the activity of the equilibrated  $\alpha$ ,  $\beta$ -mixture could be restored by simply adding sufficient acid to convert the borax to a sodium salt and boric acid. The changes of the optical activity on acidification of the borax solution were not simple but indicated the liberation of an excess of  $\alpha$ -glucose which then mutarotated to the normal value. This appeared to be a direct confirmation of Boeseken's deduction from conductivity measurements that  $\alpha$ -glucose is more favorably oriented than  $\beta$ -glucose for complex formation with boric acid. The measurement of the optical activities of sugars and certain derivatives in borax solution and the effect of acidification constitute the experimental basis of the present paper. The experimental data are discussed with respect to the formulation of the  $\alpha$ ,  $\beta$ -isomers of the sugars.

#### *Methods.*

In general the mutarotation and optical activity of the sugar in 0.5 M solution were followed in distilled water, in 0.2 M boric acid, and in 0.05 M borax (0.1 M NaOH + 0.2 M H<sub>3</sub>BO<sub>3</sub> (Kahlenberg and Schreiner, 1896)). After equilibrium of the last named solution had been attained, it was mixed with an equal volume of 0.1 N or usually 0.11 N HCl and the rotatory changes followed. A Schmidt and Haensch polarimeter reading to 0.01° and a special sodium lamp designed by West (1928) were used. No attempt was made to regulate the temperature of the solutions but the

a-glucose better than b-glucose ?

dark room remained quite constant in temperature during the day and the variations from day to day were not great.

The method of operation was to weigh the sugar into a beaker and if either boric acid or borax was to be used, it was dissolved in the water. 10 seconds before zero time, the solvent was poured over the sugar, and stirred rapidly until solution occurred.

The solution was transferred to a volumetric flask which was filled to the mark and its contents thoroughly mixed. The polarimeter tube (2 dm. tubes were used throughout) which had been previously dried was immediately filled and readings started. With practice it was found possible to obtain readings in from 2 to 5 minutes, depending on the rate of solution of the sugar used. In the acidification experiments, 10 cc. portions of the acid and 10 cc. of the equilibrated sugar borax solution were pipetted into separate beakers. At zero time, the two solutions were poured back and forth until completely mixed and the mixture was then placed in the polarimeter tube. Readings were started as soon as possible but usually 1 or 2 minutes had elapsed before the first reading could be completed. The usual precautions were observed in reading the polarimeter.

Boric acid was prepared from a c.p. sample by three crystallizations from water, followed by drying over  $\text{CaCl}_2$ . By titration in the presence of excess glucose (Weatherby and Chesny, 1926) it utilized 99.8 per cent of the theoretical quantity of  $\text{NaOH}$ .

The samples of  $\alpha$ - and  $\beta$ -glucose were prepared according to the directions of Hudson and Dale (1917). The  $\alpha$ -glucose gave an initial  $[\alpha]_D$  of  $109^\circ$  and the  $\beta$ -glucose of  $20^\circ$ . The final rotations were  $52.5^\circ$  and  $52.3^\circ$  respectively.

$\alpha$ -Methylglucoside was prepared according to the method given by Helferich and Schäfer (1926). It melted at  $167^\circ$  and showed  $[\alpha]_D = 158.1^\circ$ .

2,3,4,6-Tetramethylglucose was prepared by the methylation of glucose and subsequent hydrolysis of the methyltetramethylglucoside, according to Haworth (1915). The sample melted at  $82$ – $84^\circ$  and showed  $[\alpha]_D = 79.4^\circ$ .

Mannose was recrystallized from acetic acid according to Levene (1924). Its behavior corresponded to  $\beta$ -mannose rather than  $\alpha$ -mannose, which is supposed to be the result of this method. The final value of  $[\alpha]_D$  was  $14^\circ$ .

The remainder of the sugars were high grade commercial samples such as were used in our earlier work.

## EXPERIMENTS AND DISCUSSION.

In Fig. 1 are shown the mutarotation curves for  $\alpha$ - and  $\beta$ -glucose in water and boric acid solution (Curves I and III) and in 0.05 M

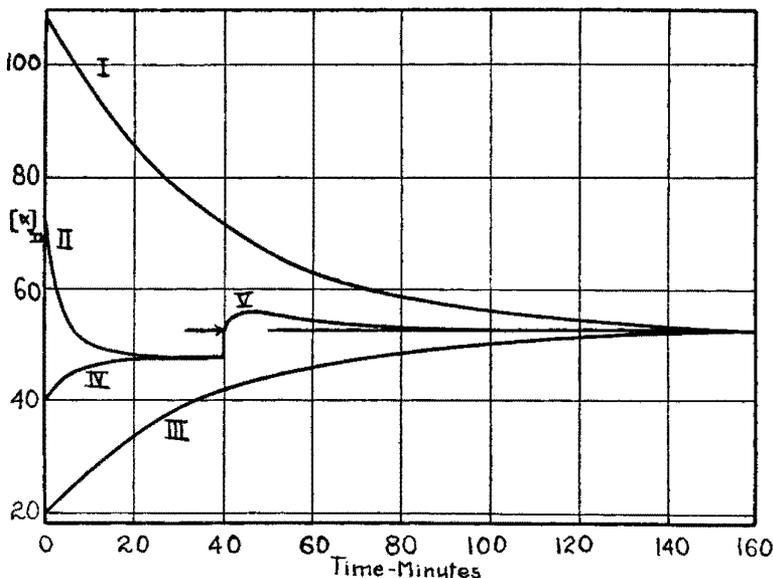


FIG. 1. Rotatory changes of  $\alpha$ - and  $\beta$ -glucose. Curve I, mutarotation of 0.5 M  $\alpha$ -glucose in distilled water or 0.2 M  $\text{H}_3\text{BO}_3$ . Curve II, 0.5 M  $\alpha$ -glucose in 0.05 M borax. Curve III, 0.5 M  $\beta$ -glucose in distilled water or 0.2 M  $\text{H}_3\text{BO}_3$ . Curve IV, 0.5 M  $\beta$ -glucose in 0.05 M borax. The arrow indicates the specific rotation of  $\alpha$ ,  $\beta$ -glucose and zero time for the curve (Curve V) obtained on acidification. The borax solution was actually acidified 24 hours after preparation.

$\text{Na}_2\text{B}_4\text{O}_7$  (Curves II and IV). The values of the specific rotation at zero time were obtained by extrapolation of the logarithmic curve of the monomolecular reaction in the usual way. It may be noted that the data upon which the curves are based conform to the monomolecular law very well, even in the borax solutions. At equilibrium glucose (0.5 M) shows  $[\alpha]_D = 52.5^\circ$  in water or boric acid and  $[\alpha]_D = 48^\circ$  in 0.05 M borax.

The fact that the glucose-borax mixtures are acid to litmus immediately after preparation excludes any change in  $[\alpha]_D$  due to alkalinity, and the truth of the statement that no irreversible changes occur is shown by the behavior of the optical activity on addition of acid to the borax solutions. This addition was made 24 hours after the preparation of the borax-glucose solution but this time has been conveniently shortened on the graph. On the addition of acid the optical activity of the glucose rose *above* the equilibrium value and then slowly fell to the value found with glucose in water or boric acid solution. It made no difference whether the solution acidified was originally prepared with  $\alpha$ -glucose or  $\beta$ -glucose; the resulting curve was the same.

Our interpretation is that in the borax solution there is present the sodium salt of a complex acid formed by the combination of glucose and boric acid. The glucose in this form has a lower optical activity than when free. On acidification this complex acid is liberated and its hydrolysis to a mixture containing an excess of  $\alpha$ -glucose causes the initial rise in activity. The fall to the normal equilibrium value is due to the mutarotation of the  $\alpha$ -glucose so liberated.

The effect of the addition of acid is shown on a larger scale in Fig. 2. In this experiment 0.1 M borax was used and a 10 per cent excess of acid was added. The extra acid hastens the hydrolysis of the free acid complex and the curve of decreasing rotation is shown in Table I to follow the monomolecular law giving a constant equal to  $0.014 \pm 0.001$  (based on common logarithms) after the 5th minute (Table I). This is the type of curve expected if an excess of  $\alpha$ -glucose had been liberated on hydrolysis of the complex.

Consideration of the acidity of the borax-glucose solutions shows that practically all of the NaOH in the solution of Fig. 1 is neutralized by the complex acid rather than by boric acid. This solution is acid to litmus. We may assume that the solution has a pH of 7. For the boric acid we may set up Equation 1.

$$(1) \quad 7 = 9.2 + \log \frac{[\text{BO}_2^-]}{[\text{HBO}_2]}$$

in which 9.2 is the value of  $\text{pK}_a$  for boric acid given by Clark (1928).

Solving, we find that  $\frac{[\text{BO}_2^-]}{[\text{HBO}_2]}$  is 0.006.  $[\text{HBO}_2] = 0.1 \text{ M}$ ; consequently  $[\text{BO}_2^-]$  must be of the order of 0.0006 M; of the total of the 0.1 M  $\text{Na}^+$  present, practically all is neutralized by the complex acid. In the following paper (Levy, 1929) it will be shown by means of freezing point measurements that the amount of glucose

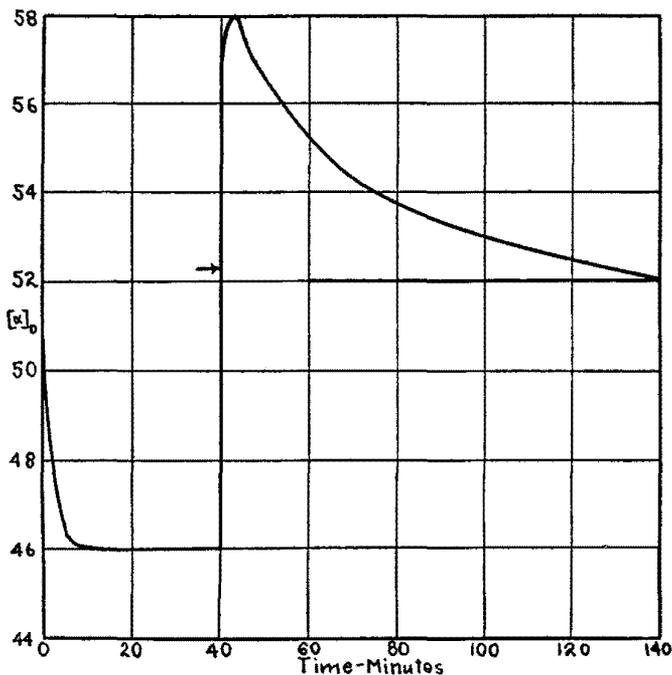


FIG. 2. Rotatory changes of  $\alpha$ -glucose in borax and the effect of acidification.  $\alpha$ -glucose 0.5 M, borax 0.1 M. The arrow indicates zero time for the addition of excess acid to the borax solution which actually occurred 24 hours after the preparation of the original solution. It also indicates the specific rotation of  $\alpha$ ,  $\beta$ -glucose in distilled water or boric acid.

combined is equal to the  $[\text{Na}^+]$  of these solutions, or 0.1 M. It is this combined glucose which is responsible for the changes of optical activity.

The interpretation of the equilibrium rotation of glucose as being simply due to a balanced reaction between  $\alpha$ - and  $\beta$ -glucose, perhaps through an intermediary present in low concentration, has

been shown experimentally to be somewhat inadequate (Lowry, 1927). However, on the basis of the older ideas, the increase in optical activity of our glucose-borax solutions on acidification may be interpreted in terms of actual amounts of  $\alpha$ - and  $\beta$ -glucose liberated. With the total amount of glucose liberated from the complex taken to be 0.1 M, the maximum activity obtained after acidification corresponded in various experiments to the liberation of a mixture containing between 55 and 66 per cent of  $\alpha$ -glucose and 45 and 34 per cent of  $\beta$ -glucose. In these experiments the latter parts of the curves obtained on acidification were extra-

TABLE I.  
*Mutarotation of  $\alpha$ -Glucose Liberated on Acidification of  
Glucose-Borax Solution.*

Glucose 0.5 M, NaOH 0.2 M,  $H_3BO_3$  0.4 M + equal volume of 0.22 N HCl.

Time after acidification.	$\alpha$ , 2 dm. tube.	$k^*$ for mutarotation.
<i>min.</i>	<i>degrees</i>	
1	5.18	
2	5.22	
3	5.22	0.000
4	5.16	0.025
5	5.17	0.0140
10	5.10	0.0135
20	4.97	0.0146
30	4.86	0.0149
40	4.83	0.0141
Final.	4.674	

\* Calculated from 5.22° as the value of  $\alpha_0$  at 2 minutes after acidification and on the basis of common logs.

polated to zero time if the values gave a satisfactory constant, but the maximum rotation was used if it could not be shown that the final change followed the monomolecular law. The extrapolated values gave the higher percentage of  $\alpha$ -glucose. These figures are considered direct evidence that a complex is formed between  $\alpha$ -glucose and borate to a greater extent than between  $\beta$ -glucose and borate (or, the existence of only one complex being assumed, that the configuration of glucose in this complex differs less from the configuration of  $\alpha$ -glucose than from  $\beta$ -glucose).

The fact that the  $\alpha$ - and  $\beta$ -isomers of glucose behave differently

towards boric acid as measured by their respective influences on the conductivity (Boeseken, 1913), as well as the experiments just described, and the interference of borate with the oxidation of the aldehyde group (Levy and Doisy, 1928) are evidences that the aldehydic hydroxyl group of the mutarotatory isomers is involved in the complex formation with borate. This is further confirmed by the fact that blocking this hydroxyl group as in  $\alpha$ - and  $\beta$ -methylglucosides eliminates the effect on the conductivity (Boeseken and Couvert, 1921), and by the experiments of Table II which show that borate has only a slight effect on the specific rotation of  $\alpha$ -methylglucoside.

While Table II shows a slight decrease in the optical activity of  $\alpha$ -methylglucoside in the presence of borate, none of the changes characteristic of glucose occur following acidification, the rotation

TABLE II.  
*Effect of Borax on the Rotatory Power of  $\alpha$ -Methylglucoside.*

The glucoside was 0.5 M in each solution except Solution 4.

Solution No.	Solvent.	$\alpha$	$[\alpha]_D$
		2 dm. tube.	
		<i>degrees</i>	<i>degrees</i>
1	H <sub>2</sub> O	30.692	158.08
2	0.2 M H <sub>3</sub> BO <sub>3</sub> .	30.696	158.10
3	0.05 M Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> .	30.361	156.32
4	10 cc. Solution 3 + 10 cc. 0.1 N HCl.	15.361	158.24

rising immediately to the final value given. This leads to the conclusion that these changes with glucose must have been dependent on the presence of the free aldehydic hydroxyl group.

Boeseken (1913) from his systematic studies of the effect of various hydroxyl-containing compounds on the conductivity of boric acid has concluded that complex formation occurs with such a compound when two hydroxyl groups are situated on adjacent carbon atoms and on the same side of the plane in which these carbon atoms lie. If the aldehydic hydroxyl group is involved in the complex formation, it follows from this generalization that the hydroxyl group of carbon atom 2 is also involved. This is the basic principle underlying the formulæ for  $\alpha$ - and  $\beta$ -isomers suggested by Boeseken for glucose and other sugars.

If Boeseken's principal assumption is correct, 2,3,4,6-tetra-

methylglucose should not combine with boric acid because the hydroxyl group of carbon atom 2 is blocked. Irvine and Steele (1915) tested the substance and concluded that the methylated glucose increased the conductivity of boric acid quite as much as did glucose and that the conductivity changed in the same manner as the rotation during mutarotation. However, Boeseken and Couvert (1921) repeated these experiments in exactly the same way and their results show that tetramethylglucose does not combine with boric acid, so that, in view of our studies which show no evidence of combination between the methylated sugar and borax, the apparent contradiction of Irvine and Steele's data is probably due to an experimental factor.

TABLE III.  
*Rate of Oxidation of Tetramethylglucose by Alkaline Iodine Solution.*

Time.	Per cent oxidation.	
	$\text{Na}_2\text{CO}_3, \text{NaHCO}_3$ pH = 10	$\text{Na}_2\text{CO}_3, \text{H}_3\text{BO}_3$ pH = 10.1
<i>min.</i>		
2	55.2	64.7
5	76.2	81.8
10	88.2	93.7
20	98.2	99.3
40	100.2	100.3
60	100.5	100.5
120	100.7	100.7

Table III gives data on the oxidation of tetramethylglucose by alkaline iodine solutions, the experiments being performed in the manner described by Levy and Doisy (1928). The slightly greater rate of oxidation in the borate-carbonate buffer is due to the slightly greater alkalinity as indicated by the pH of the buffer. The difference is perhaps exaggerated because of the unavoidable experimental error in following such a rapid reaction. Since these data show that borate does not interfere with the oxidation, it is improbable that a complex is formed.

In addition to the evidence in Table III, the optical activity of the methylated sugar is but slightly affected by borax under the conditions which gave a marked change in the optical activity of

glucose and the solution shows very little, if any, of the changes on acidification characteristic of glucose (Table IV).

The evidence so far submitted indicates that the glucosidic hydroxyl group and one of the chain hydroxyl groups are involved in complex formation. The particular chain hydroxyl group involved is probably the one adjacent to the aldehyde group (carbon atom 2). The results of acidification show that  $\alpha$ -glucose is the more favorably oriented of the two forms and should be given the *cis* formulation in the ring formulations of sugars adopted by Boeseken (1913) to bring out this relationship, and more recently advocated by Goodyear and Haworth (1927) and by Haworth (1929) to show the relationship of the sugars to pyran and furan. If we adopt Haworth's formulations of glucose as a derivative of

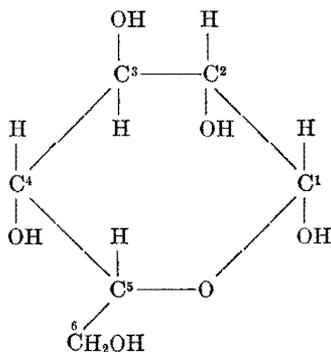
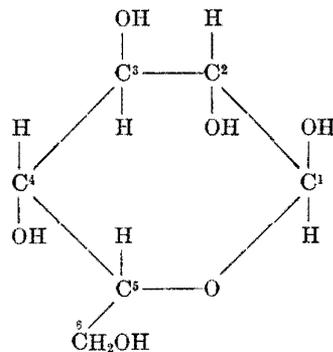
TABLE IV.

*Effect of Borax on the Optical Activity of 2,3,4,6-Tetramethylglucose.*

The concentration of tetramethylglucose was 0.5 M except in Solution 4.

Solution No.	Solvent.	Initial.		Final.		<i>k</i> for mutarotation.
		$\alpha$	$[\alpha]_D$	$\alpha$	$[\alpha]_D$	
1	H <sub>2</sub> O	21.89	92.67	18.76	79.42	0.013
2	0.2 M H <sub>3</sub> BO <sub>3</sub> .	21.83	92.42	18.75	79.36	0.014
3	0.05 M Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> .	20.09	85.05	18.57	78.63	0.18
4	10 cc. Solution 3 + 10 cc. 0.1 N HCl.	9.33	78.83	9.37	79.33	

pyran,  $\alpha$ -glucose is represented by Formula I and  $\beta$ -glucose by Formula II. These are the formulæ adopted by Boeseken (1913)

I.  $\alpha$ -Glucose.II.  $\beta$ -Glucose.

and correspond to those advocated by Pictet (1920) and Michaelis (1913) for these isomers from entirely independent evidence.

Hudson (1909) has shown that the  $\alpha$ - and  $\beta$ -isomers of a sugar represent the two possible configurations of carbon atom 1 as an asymmetric center. If the formulæ deduced above are correct, then in  $\alpha$ -glucose carbon atom 1 has the same configuration as carbon atom 2, which is *d* (Wohl and Freudenberg, 1923), and in  $\beta$ -glucose the opposite, or *l*. According to Hudson's nomenclature all  $\alpha$ -aldose sugars of the *d* series (genetically related to *d*-glucose) will have the same configuration of carbon atom 1, or the *d* configuration according to the above formulæ, and all  $\beta$ -aldose sugars of this series the opposite or *l* configuration. Similarly, all  $\alpha$ -aldoses of the *l* series will have the same configuration of carbon atom 1, which will be opposite to that of the  $\alpha$ -sugars of the *d* series, or, according to the above formulæ, the *l* configuration, and the  $\beta$ -sugars of the *l* series will have the *d* configuration of carbon atom 1. These requirements are fully met by the conclusions drawn from experiments on the optical activity of various sugars in borax and the effect of acidification which will be presented below.

The curves of Fig. 3 represent the mutarotation of the various sugars in borax solution and the effect of acidification. In some cases (lactose and maltose) mutarotation was so rapid that it could not be followed accurately and in these cases the mutarotation curve is omitted. It is noteworthy that these solutions were quite alkaline and freezing point data show a lesser extent of complex formation than with other sugars. The main point of interest, however, is the behavior of the optical activities on acidification. In every case except mannose, the more dextrorotatory form is liberated in excess of the equilibrium normally expected. The case of rhamnose is not particularly marked, although the data obtained do show the liberation of the dextro form. With mannose the more levorotatory isomer is liberated on acidification. The stereochemical formulation of each aldose used has for carbon atom 2 the *d* configuration except mannose, in which carbon atom 2 has the *l* configuration. In the form which combines most readily with borate, as shown by its liberation when the complex is decomposed, carbon atoms 1 and 2 are presumed to have the same configuration. Consequently the more dextrorotatory form of the

pair of isomers for each sugar has the *d* configuration for carbon atom 1. This relation is made apparent in Table V. Fructose is not included since it is a keto sugar and the principle of optical superposition precludes the application of relationships determined

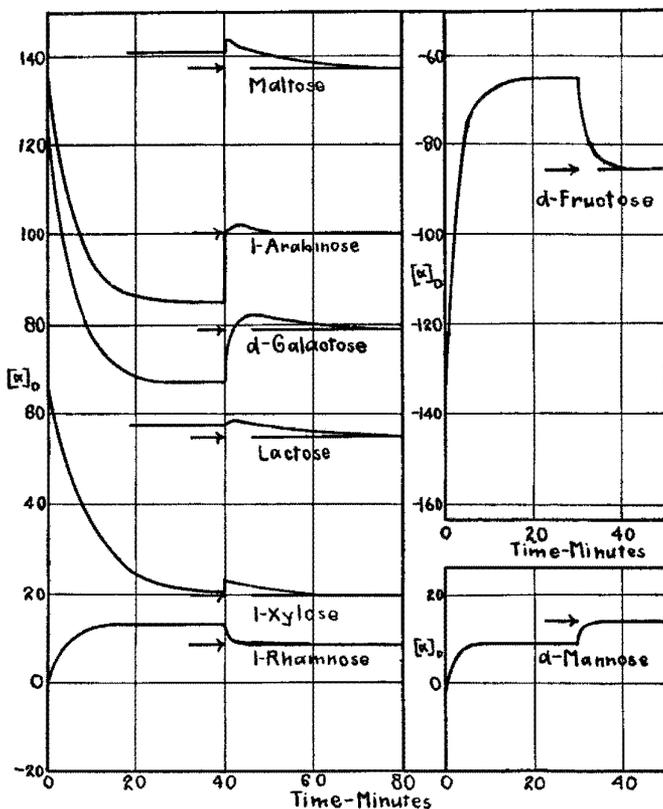


FIG. 3. Rotatory changes of various sugars in borax and the effect of acidification. The sugar in each case is 0.5 M and the borax 0.05 M. The arrows indicate zero time for the acidification (24 hours after preparation of the sugar-borax solution) and the specific rotation of the sugar in distilled water or 0.2 M boric acid at equilibrium.

for the aldoses (Hudson, 1909). In addition complex formation could occur between carbon atoms 1 and 2 or 2 and 3 just as with the acetone derivatives of fructose (Anderson, Charlton, Haworth, and Nicholson, 1929) and one cannot be sure what configurational

significance the fact that the dextrorotatory form is liberated on acidification may have.

The configurations given in Table V agree with those adopted by Boeseken (1913), Boeseken, Kerstjens, and Klammer (1916), and Boeseken and Couvert (1921) from conductivity data, except for mannose. With this sugar the latter authors found that the conductivity increases as the  $\alpha$ -isomer is formed. It seems to the present writers that the method described in this paper is subject to less disturbing influence. The conductivity of the solutions of sugars and boric acid depends on two factors: (1) The amount of complex acid formed which is the criterion by which the stereo-

TABLE V.  
*Configuration of Carbon Atom 1 of Aldoses.*

Sugar.	[ $\alpha$ ] <sub>D</sub> of form liberated in excess.	[ $\alpha$ ] <sub>D</sub> of other form.	Configuration.	
			Carbon atom 2.	Dextro form of carbon atom 1
	<i>degrees</i>	<i>degrees</i>		
<i>d</i> -Glucose.....	109	20	<i>d</i>	<i>d</i>
<i>d</i> -Mannose.....	-17	34	<i>l</i>	"
<i>d</i> -Galactose.....	140	53	<i>d</i>	"
<i>l</i> -Arabinose.....	184	76	"	"
<i>l</i> -Xylose.....	92	-20	"	"
<i>l</i> -Rhamnose.....	54	-7.7	"	"
Maltose.....	168	11.8	"	"
Lactose.....	86	35.0	"	"

chemical relations should be judged, and (2) the dissociation of the acid formed, which is not necessarily related to the configuration of carbon atom 1. The increase in conductivity is a measure of both factors, whereas the optical method described here depends only on the amount of complex acid formed. It is felt therefore that the configuration presented for mannose is more reliable than Boeseken's configuration. In addition the present configuration is consistent with Hudson's (1909) generalizations.

#### CONCLUSIONS.

1. The optical activities of sugars differ in borax solution from the optical activities in water.

2. The expected activity is restored when the base of the borax is neutralized by a strong acid.

3. The acidified solutions undergo rotatory changes before reaching equilibrium, which can be correlated with the configuration of carbon atoms 1 and 2 of the aldehyde sugars.

4. The data obtained have been used to show that the more dextrorotatory of the  $\alpha$ - and  $\beta$ -isomers of each aldose sugar has the *d* configuration for the aldehydic carbon atom.

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