RP441

A STUDY OF THE DELTA LACTONES FORMED BY THE OXIDATION OF ALDOSES WITH BROMINE WATER

By Horace S. Isbell

ABSTRACT

Inasmuch as the free sugar acids are converted to their barium salts by barium carbonate but their lactones are left unaltered, this substance may be used for determining whether the free acid is formed at any time during the course of the oxidation of the aldoses with bromine water. If the oxidation product were the free acid it would be neutralized by the barium carbonate, but if it were the lactone it would not be changed. It has been found in some cases that only 1 or 2 per cent of the oxidation product is neutralized, which proves that the sugar acids are not produced in the course of the oxidation of the aldose sugars by bromine water in the presence of barium carbonate. The products produced are neutral substances which on standing in aqueous solution develop acidity. Measurements of the optical rotations, as well as the changes in acidity, indicate that delta lactones are formed by the bromine oxidation of α- and β-*d*-mannose, α- and β-*l*-rhamnose, α- and β-lactose, β-cellobiose, and β-maltose.

The beta forms of the sugars are more easily oxidized than the alpha, but delta lactones are produced from both. The fact that delta lactones are obtained from α- and β-*d*-mannose (as well as from the other sugars) is at least circumstantial evidence supporting the 1, 5 ring structure for the substances investigated.

CONTENTS

I. INTRODUCTION

In a previous publication C. S. Hudson and the writer [1] reported some observations which indicate that delta lactones are formed directly from certain aldose sugars by oxidation with bromine water. This experimental evidence requires the abandonment of the generally accepted concept that the aldehyde form of the sugar is oxidized to the monocarboxylic acid, in favor of a new hypothesis, which, stated briefly, is that the cyclic forms of the normal sugars give delta lactones by oxidation with bromine water. Since the evidence for this was derived solely from the optical rotations of the sugars during the oxidation, it is desirable to obtain more direct evidence in support of the new hypothesis.

The lactones of the sugar acids are neutral substances which, on standing in aqueous solution, are partially hydrolyzed to the corresponding acids. As a result of this hydrolysis the hydrogen-ion concentration of a freshly prepared solution of the lactone increases on standing; whereas the hydrogen-ion concentration of the corresponding acid decreases on standing because of lactone formation. Since

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[1] Isbell and Hudson, B. S. Jour. Research, vol. 8 (RP418), p. 327, 1932.

the oxidation product reported in the previous paper was dissolved in a solution buffered with sodium acetate, it was not possible to follow its hydrolysis by means of hydrogen-ion determinations. This objectionable feature has been overcome by selecting an insoluble buffer (barium carbonate), the excess of which may be removed by filtration after the oxidation is complete. The experimental conditions are such that any acid which may be present is neutralized by the barium carbonate, but the lactone is not saponified. It will be shown in Section III that in some cases only 1 or 2 per cent of the oxidation product is neutralized by the barium carbonate. This is direct proof that the oxidation product is not the acid, as previously believed. The hydrogen-ion concentrations, as well as the specific rotations of the products obtained from α- and β-*d*-mannose, α- and β-*l*-rhamnose, α- and β-lactose, β-cellobiose, and β-maltose, indicate that these substances give delta lactones on bromine oxidation.

II. EXPERIMENTAL METHOD

In order to study the substance produced by the oxidation of a sugar with bromine water, it is highly desirable that the sugar be converted as completely as possible into the primary oxidation product. When the reaction is conducted according to the method previously used [2] a substantial portion of the sugar is not oxidized. The presence of the unoxidized sugar is particularly objectionable because it is not known whether this residual sugar is in equilibrium immediately after the oxidation. If it is not in equilibrium, the observed mutarotation is the resultant of the changes due to the unoxidized sugar and those due to the oxidation product. Obviously this complication would be eliminated if the sugar were converted completely into the oxidation product. The experimental conditions must be such that the delta lactones formed in the reaction may be identified. Previously Bunzel and Mathews [3] showed that the bromine oxidation of the aldoses is retarded by the presence of a small quantity of acid. Since the delta lactones are saponified very rapidly by dilute alkali, the reaction must be maintained on the acid side of neutrality. A suspension of calcium or barium carbonate in water, saturated with carbon dioxide, may be used as a means for maintaining the reaction of a solution nearly neutral, but on the acid side of neutrality. As first shown by Clowes and Tollens [4] the aldose sugars are oxidized rapidly by bromine water in the presence of calcium carbonate. Although they, and subsequent investigators using the same buffer, obtained the calcium salt rather than the unchanged oxidation product, their experimental conditions were such that, even though the lactone were formed, it would have been hydrolyzed and finally neutralized with calcium carbonate. The writer has found that delta lactones are formed by the oxidation of the aldoses by bromine water in the presence of calcium carbonate and carbon dioxide. When this buffer is employed the solution of the delta lactone contains considerable calcium bicarbonate, which is objectionable. The use of barium carbonate instead of calcium carbonate gives a product which contains less bicarbonate, and consequently this buffer was selected.

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[2] See footnote 1, p. 615.
[3] Bunzel and Mathews, J. Am. Chem. Soc., vol. 31, p. 464, 1909.
[4] Clowes and Tollens, Ann., vol. 310, p. 166, 1900.

Barium carbonate in the presence of carbon dioxide maintains the reaction of the solution slightly acid, in which condition the interchange between the alpha and beta forms of the sugars is slow and the oxidation is relatively rapid. The preliminary results of an investigation [5] of the reaction rates of the alpha and beta forms of the principal sugars has shown that the beta forms are oxidized much more readily by bromine water than the corresponding alpha isomers. By taking advantage of this fact it is possible in some cases to convert over 95 per cent of the sugar to the lactone in five minutes.

The crystalline sugar is added to a suspension of barium carbonate in bromine water and the oxidation is allowed to continue in the presence of carbon dioxide for a predetermined time. The barium carbonate neutralizes the hydrobromic acid formed from the bromine and thus maintains a nearly neutral reaction. Since the barium carbonate in the presence of carbon dioxide is difficultly soluble and nearly neutral it does not appreciably decompose the lactone, which remains in solution as such.[6] When the oxidation is nearly complete the excess bromine is removed by shaking the solution with linseed oil, and the excess barium carbonate is separated by filtration. The solution thus obtained does not contain free bromine or a buffering agent. Therefore the hydrolysis of the lactone may be followed by hydrogen-ion determinations. The amount of the oxidation product neutralized by the barium carbonate may be estimated from the difference in the quantity of the oxidation product theoretically formed, as calculated from the weight of the sugar actually oxidized, and the quantity of lactone actually present in the solution, as determined by titration with standard alkali.

TABLE 1.—*Oxidation of aldoses by bromine water in the presence of barium carbonate*

Aldose used	Grams of sample in 200 ml [a]	Time (in minutes) allowed for oxidation	Per cent of sample not oxidized	Per cent of sample oxidized	Moles of product formed by oxidation [b]	Moles of unchanged lactone in oxidation product [c]	Per cent of oxidation product present as lactone	Per cent of oxidation product neutralized by $BaCO_3$
α-*d*-mannose	9	10	20.0	80.0	0.0400	0.0396	99.0	1.0
β-*d*-mannose	9	5	2.9	97.1	.0485	.0478	98.6	1.4
α-*l*-rhamnose (hydrate)	9.1	15	9.0	91.0	.0455	.0448	98.5	1.5
β-*l*-rhamnose	8.2	10	4.4	95.6	.0478	.0466	97.5	2.5
α-lactose (hydrate)	18	10	39.9	60.1	.0300	.0228	76.0	24.0
β-lactose	17.1	5	3.4	96.6	.0483	.0432	89.4	10.6
β-cellobiose	17.1	10	2.3	97.7	.0488	.0364	74.6	25.4
β-maltose	18	4	2.4	97.6	.0488	.0470	96.3	3.7

[a] The samples correspond to 0.05 mole.

[b] This value is based on the assumption that the sugar was converted quantitatively into the lactone, and consequently the numerical value is the same as the amount of sugar actually oxidized.

[c] This value was determined by the amount of alkali necessary to effect the saponification of the oxidation product.

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[5] This investigation, which was outlined briefly in the Journal of the American Chemical Society, vol. 54, p. 1692, 1932, is being conducted with the assistance of W. W. Pigman.

[6] The solution resulting from the oxidation contains barium bromide and the delta lactone. The barium bromide may be removed with silver sulphate, leaving a solution of the nearly pure delta lactone. This new method for obtaining delta lactones is being applied to certain sugar acids which have not given crystalline delta lactones by other methods.

III. DISCUSSION OF RESULTS

The results given in Table 1 were obtained in the manner outlined in the previous paragraph. Since there is a considerable difference in the reaction rates of the sugars, a corresponding length of time was allowed for each oxidation, as shown in the third column of Table 1. As may be seen from the percentage of the sample oxidized, column 5, the beta forms of the sugars are attacked more rapidly than the corresponding alpha isomers. The amount of each oxidation product was calculated by multiplying the moles of the sugars used (0.05) by the percentage of the sugar oxidized. Before considering the amount of the unchanged lactone in the oxidation product, column 7, and the percentage of the oxidation product neutralized by barium carbonate, column 9, it should be recalled that the oxidation was conducted in the presence of barium carbonate and that the free sugar acids are quickly neutralized by shaking with this substance, but the delta lactones are not. The moles of unchanged lactone in the oxidation product, column 7, were determined by titration after the reaction was stopped and the barium carbonate removed. The difference between the total amount of the product formed by oxidation, column 6, and the amount of the unchanged lactone, column 7, gives the amount of the oxidation product neutralized by barium carbonate. This is expressed in per cent in column 9 of Table 1. The amount of the oxidation product neutralized varies with different sugars. The fact that in some cases only 1 or 2 per cent of the products was neutralized by barium carbonate is particularly significant because any free acid which might be formed, perhaps by the oxidation of the open-chain form of the sugar, would be neutralized by the barium carbonate. Surprisingly small amounts of the lactones derived from mannose and rhamnose were saponified, but more decomposition was found for the lactones derived from lactose, cellobiose, and maltose. Lactobionic, cellobionic, and maltobionic lactones are more readily hydrolyzed than the delta lactones of mannonic and rhamnonic acids. It should be recalled again that the oxidation product is exposed during the oxidation to barium carbonate which neutralizes any acid which may be formed directly from the sugar or by hydrolysis. The differences in the amounts of decomposition are probably due to differences in the rates of hydrolysis, together with the fact that when the oxidation was allowed to continue for longer periods, there was more opportunity for the hydrolysis of the lactone and the neutralization of the acid thus formed.

The oxidation of the alpha and beta forms of mannose is of interest because of the uncertainty regarding their structure.[7] [8] [9] If a sugar containing a 1, 5 ring is oxidized to give a delta lactone we would expect a sugar containing a 1, 4 ring to give a gamma lactone.[10] Hence if α-*d*-mannose contained a 1, 4 ring we would expect it to give a gamma lactone. The gamma lactones differ from the delta lactones in that they are more slowly hydrolyzed, requiring weeks to reach equilib-

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[7] Hudson, J. Am. Chem. Soc., vol. 52, p. 1689, 1930.

[8] Haworth, Hirst, Streight, Thomas, and Webb, J. Chem. Soc., p. 2636, 1930.

[9] Isbell, B. S. Jour. Research, vol. 7 (RP392), p. 1115, 1931.

[10] Some 1, 4 methylated sugars are being made with the object of submitting them to bromide oxidation in order to determine whether they give gamma lactones or acids. The method is being applied also to the free aldehyde forms of certain acetylated sugars. By oxidation with bromine water the free aldehyde form should give the free acid, but its semiacetal form may give the ethyl esters.

rium. If a gamma lactone were formed from either *α*- or *β*-*d*-mannose the changes in acidity and optical rotation would be so gradual that they would not be apparent in the course of several hours.

The changes in the specific rotations of the oxidation products derived from *α*- and *β*-*d*-mannose are given in Curves I and II of Figure 1; and the corresponding increases in acidity are illustrated in Curves III and IV of the same figure. As illustrated in Curve II, the product derived from *β*-*d*-mannose gives an initial specific rotation of about +110 which decreases to about +30 in 24 hours and thereafter slowly increases. According to Nef[11] the delta mannonic lactone gives $[\alpha]^{20}_{D} = +111.1$ initially, which decreases to +28 in 24 hours, and thereafter slowly increases. The values for the specific rotations of the product derived from *α*-*d*-mannose (Curve I) are lower than those from *β*-*d*-mannose (Curve II) because the sample of *α*-*d*-mannose was oxidized less completely. The oxidation of the sample of *β*-*d*-mannose was 97 per cent complete, but the sample of *α*-*d*-mannose was oxidized only to the extent of 80 per cent. The rotation of the lactone formed from *α*-*d*-mannose may be calculated from the observed rotation by assuming that the solution contains only the lactone and the unoxidized sugar. The specific rotation of the unoxidized sugar, when the first reading was taken, was somewhere between the specific rotation of *α*-*d*-mannose and the equilibrium rotation. If we assume that the rotation of this unoxidized sugar is +30 (the rotation of *α*-*d*-mannose) the calculated specific rotation of the lactone formed is +104. Assuming that the specific rotation of the unoxidized sugar is +14.6 (the equilibrium rotation) then the calculated specific

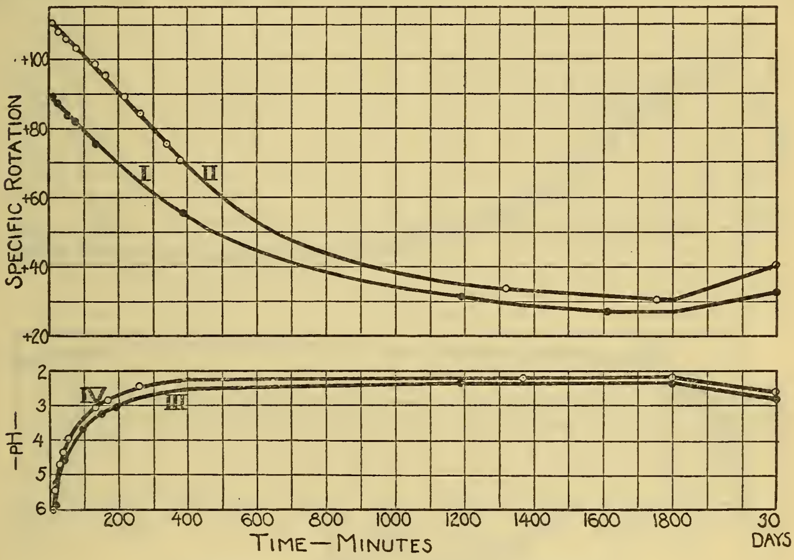

FIGURE 1.—*Bromine oxidation of mannose*

I, changes in the specific rotation of the product derived from *α*-*d*-mannose.
II, changes in the specific rotation of the product derived from *β*-*d*-mannose.
III, changes in acidity (pH) of the product derived from *α*-*d*-mannose.
IV, changes in acidity (pH) of the product derived from *β*-*d*-mannose.

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[11] Nef, Ann., vol. 403, p. 312, 1914.

rotation of the lactone formed is +107. This value is near to the specific rotation of the delta lactone (+111) although somewhat lower. Hence it appears that the delta lactone is derived from both *α*- and *β*-*d*-mannose. This conclusion is further supported by the changes in pH shown in Curves III and IV. The changes are relatively rapid in both cases and show that the acidity increases on standing, reaching a maximum value in several hours. This increase in acidity indicates that the oxidation product is hydrolyzed to give an acid. A small decrease in acidity occurs on long standing. This is caused by the formation of the gamma lactone from the quasi equilibrium mixture of the free acid and the delta lactone, a reaction characteristic of acids which are capable of forming gamma and delta lactones.[12] The slightly lower acidities found for the product derived from *α*-*d*-mannose (Curve III) are caused by the less complete oxidation of this sugar giving less lactone, which in turn gives less of the acid. The differences just mentioned are small and attention is directed again to the close similarity of the curves, which clearly indicate that the same substance is present in both cases.

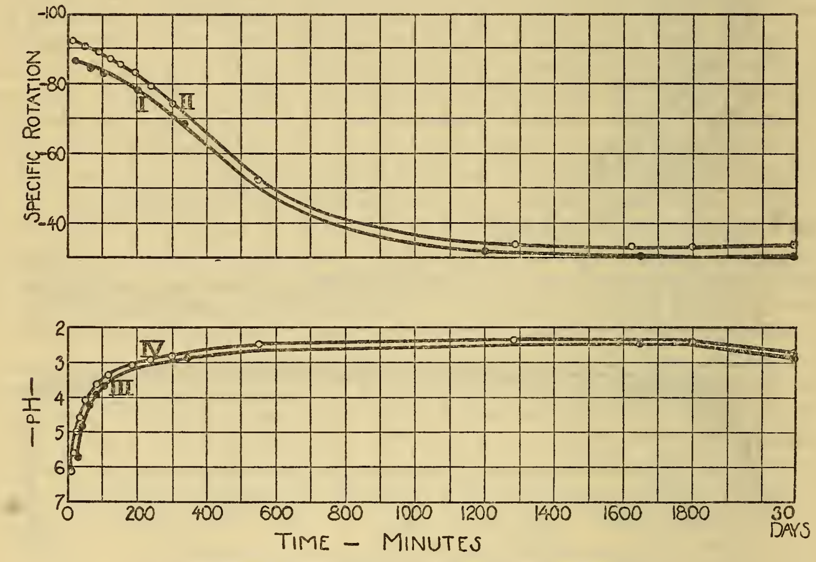

FIGURE 2.—*Bromine oxidation of rhamnose*

I, changes in the specific rotation of the product derived from *α*-*l*-rhamnose.
II, changes in the specific rotation of the product derived from *β*-*l*-rhamnose.
III, changes in acidity (pH) of the product derived from *α*-*l*-rhamnose.
IV, changes in acidity (pH) of the product derived from *β*-*l*-rhamnose.

Since the *α*- and *β*-forms of rhamnose react more slowly than the corresponding forms of mannose the oxidations of these sugars were allowed to continue 10 and 15 minutes, respectively. The results are shown in Figure 2. A comparison of the specific rotations of the product derived from *α*-*l*-rhamnose (Curve I), with the specific rotations of the product derived from *β*-*l*-rhamnose (Curve II), reveals a marked similarity between the two which indicates that the same product is obtained from *α*-*l*-rhamnose as from *β*-*l*-rhamnose. The

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[12] Levene and Simms, J. Biol. Chem., vol. 65, p. 31, 1925.

oxidation product from β-*l*- rhamnose gave $[\alpha]^{20}_{D} = -92.5$, 15 minutes after the addition of the bromine, changing in 24 hours to -34. Jackson and Hudson[13] report that the delta rhamnonic lactone gives $[\alpha]^{23}_{D} = -98.4$ (7 minutes after solution), changing to -61 in 6 hours and thereafter to -30 in 11 weeks. Since the sample of β-*l*-rhamnose was oxidized only to the extent of 95.6 per cent, the observed rotation is sufficiently near to that reported by Jackson and Hudson to indicate that it is the delta lactone. Further evidence in support of this conclusion is given in Curves III and IV which show the changes in pH of the oxidation products obtained from α- and β-*l*-rhamnose. In each case the acidity increases on standing, which shows that an acid is formed from the oxidation product.

The specific rotations of solutions obtained after oxidations of α- and β-lactose are illustrated by Curves I and II in Figure 3. The oxidation of β-lactose was 96.6 per cent complete in 5 minutes, but the oxidation of α-lactose was only 60.1 per cent complete in 10 minutes. The values for the specific rotations of the oxidation product derived from α-lactose are larger than those obtained from β-lactose because the sample of α-lactose was not completely changed into the delta lactone. As may be seen from Curves III and IV of Figure 3 the oxidation product from both α- and β-lactose is hydrolyzed relatively fast. Since it is hydrolyzed so easily, there is some decomposition during the preparation of the sample and the acid formed thereby is neutralized with barium carbonate. Inasmuch as the oxidation of α-lactose was allowed to continue longer than the oxidation of β-lactose the amount of the oxidation product neutralized, as given in Table 1, is greater.

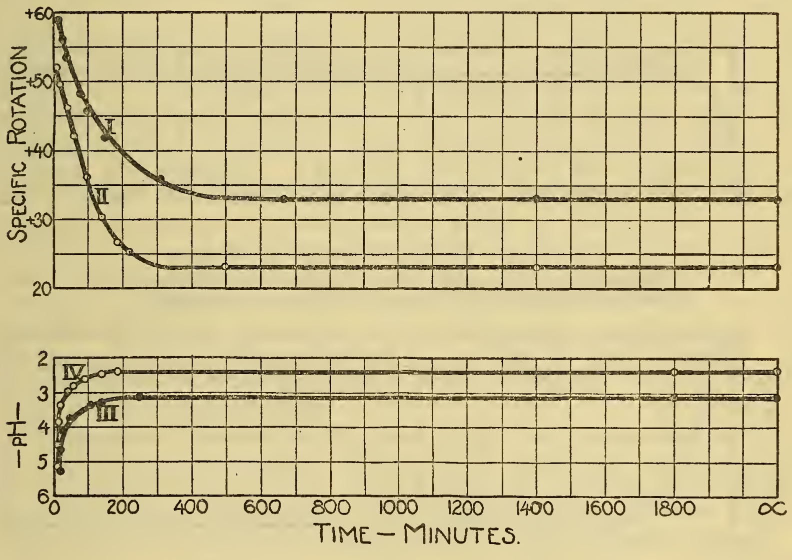

FIGURE 3.—*Bromine oxidation of lactose*

I, changes in the specific rotation of the product derived from α-lactose.
II, changes in the specific rotation of the product derived from β-lactose.
III, changes in acidity (pH) of the product derived from α-lactose.
IV, changes in acidity (pH) of the product derived from β-lactose.

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[13] Jackson and Hudson, J. Am. Chem. Soc., vol. 52, p. 1273, 1930.

The changes in the specific rotation of the oxidation product derived from β-cellobiose are illustrated in Curve I of Figure 4. The corresponding changes in pH are shown in Curve II of the same figure. The changes are rapid, indicating that the lactone is easily hydrolyzed. This accounts for the relatively large amount of cellobionic lactone which was hydrolyzed during the oxidation of the sugar and subsequently neutralized with barium carbonate, as given in the right-hand column of Table 1.

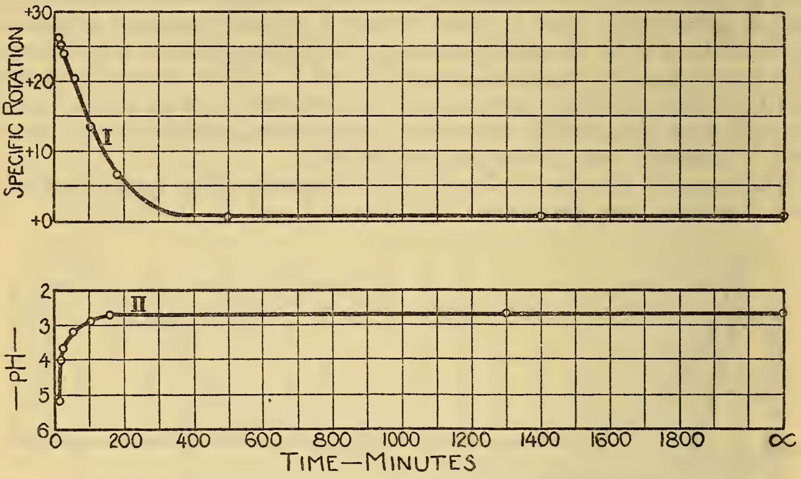

Figure 4.—*Bromine oxidation of cellobiose*

I, changes in the specific rotation of the product derived from β-cellobiose.
II, changes in acidity (pH) of the product derived from β-cellobiose.

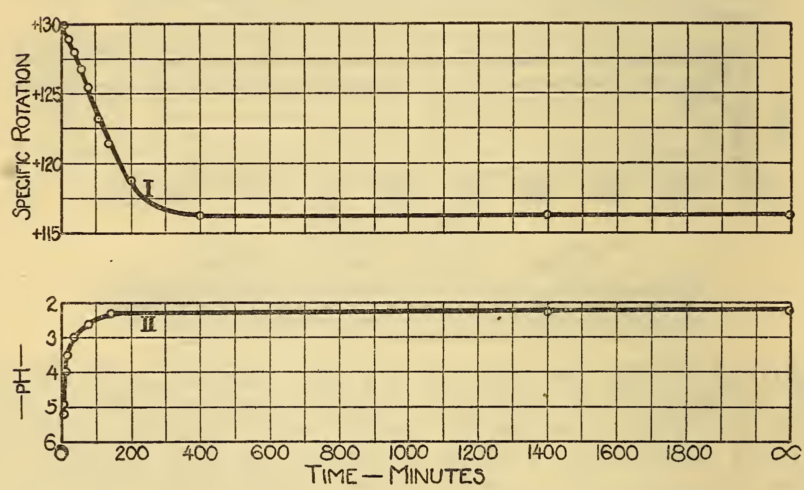

Figure 5.—*Bromine oxidation of maltose*

I, changes in the specific rotation of the product derived from β-maltose.
II, changes in acidity (pH) of the product derived from β-maltose.

The changes in the specific rotation and pH of the oxidation product derived from β-maltose are shown in Curves I and II of Figure 5. Maltobionic lactone is easily hydrolyzed, but since the oxidation of maltose was allowed to continue for only four minutes the hydrolysis of the lactone as indicated by the right-hand column of Table 1 was less than that found with cellobiose and lactose.

IV. EXPERIMENTAL DETAILS

The bromine oxidation of the sugar was conducted in the presence of 30 g of barium carbonate which was placed in a 1-liter flask and a predetermined quantity of water added. The amount of water used was such that the volume of the solution was 200 ml after the addition of 0.05 mole of the sugar and the subsequent oxidation thereof. The suspension of the barium carbonate in water was saturated with carbon dioxide at 20° C. and, after the addition of 10 ml of bromine, 0.05 mole of the finely powdered crystalline sugar was added. The flask was shaken and cooled in running water to maintain the temperature at 20° C. After 3, 4, 9, or 14 minutes, depending upon the sugar, the flask was cooled in ice water for 1 minute, during which the temperature decreased to about 12° C. The oxidation was then stopped by the addition of 50 ml of linseed oil dissolved in 50 ml of benzene, the flask being continuously cooled by ice water. The solution became colorless in about 15 seconds, showing that all the bromine was removed. The flask was shaken for one minute more, and then the cold solution was filtered. Sometimes a second filtration was necessary in order to remove any oil which passed through the first filter. A colorless filtrate was obtained which was used for following the optical rotations and hydrogen-ion concentrations.

After standing for several hours the solution of the oxidation products obtained as given above frequently developed a small amount of color, which appears to be due to free bromine. This was removed by shaking with a drop of linseed oil and filtration prior to all analytical work.

The optical rotations were read in a 2 dm water-jacketed tube on a Bates saccharimeter. The specific rotations were calculated for the anhydrous lactones, since it may be assumed that the sugar is converted into the lactone. The weights of the lactones are only slightly different from the weights of the corresponding sugars.

The hydrogen-ion determinations were made with a quinhydrone electrode in the usual manner. The samples of the oxidation products were carefully freed from all bromine and kept in a thermostat at 20° C. The results are expressed in the curves in terms of pH.

The amount of unoxidized sugar (reducing substance) remaining in the solution after the oxidation was determined by Scales's method[14] and, as given in Table 1, the percentage of the sample oxidized, as well as the equivalents of lactone formed by oxidation, were calculated from the amount of unoxidized sugar. The reagents used in determining the amount of unoxidized sugar were standardized by analyzing a solution containing a known amount of the sugar under investigation.

The equivalents of lactone present in the solution of the oxidation product were obtained by titration of the solution with standard alkali. A sample of the solution (10 ml) was heated to boiling to remove the carbon dioxide and 25 ml of *N*/10 barium hydroxide added. After a few minutes the excess alkali was titrated with

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[14] Scales, J. Ind. Eng. Chem., vol. 11, p. 747, 1919.

N/10 sulphuric acid, using phenolphthalein as an indicator. The results were corrected for the small amount of barium bicarbonate or other basic constituents which remained in the solution. This was determined from a blank experiment which was conducted in manner analogous to the oxidation of the sugar, except that 9 g of barium bromide was substituted for the sugar. The barium carbonate, carbon dioxide, bromine, and linseed oil were added as before. A 10 ml sample of the final solution was heated to boiling with 5 ml of *N*/10 sulphuric acid and the excess acid was subsequently determined by titration with standard alkali. The 10 ml sample of the blank neutralized 0.4 ml of *N*/10 acid, and hence this correction was applied to the titrations of the oxidation products derived from the sugars.

A portion of the solution prepared for the blank run was used also for showing that in the absence of the product obtained by the oxidation of the sugars no changes in the hydrogen-ion concentration occurred on standing. The pH of the solution remained constant at 6.4.

V. ACKNOWLEDGMENT

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