

# THE OXIDATION OF SUGARS. I. THE ELECTROLYTIC OXIDATION OF ALDOSE SUGARS IN THE PRESENCE OF A BROMIDE AND CALCIUM CARBONATE

By Horace S. Isbell and Harriet L. Frush

## ABSTRACT

A new method has been devised for the preparation of calcium salts of the sugar acids in which the sugar is electrolytically oxidized in the presence of calcium carbonate and a bromide. The sugar is oxidized in manner analogous to the well-known bromine oxidation, but since only a small quantity of bromide is used as a catalyst, the new method has the decided advantage that the reaction product does not contain any objectionable substances derived from the oxidant. On account of this, the separation of the product is greatly simplified, as shown by the fact that calcium gluconate, galactonate, mannonate, rhamnonate, and arabonate are easily crystallized on evaporation of the solutions resulting from the oxidation of the respective sugars. The preparation of the calcium salts of xylonic, lactobionic, and maltobionic acids is also given.

The raw materials, with the exception of the bromide, which is used only in small quantities as a catalyst, are relatively cheap. This fact, in conjunction with the high current efficiency and high yield, should make the method of considerable commercial value for the preparation of calcium gluconate and related products.

## CONTENTS

	Page
I. Introduction.....	1145
II. A new process for the manufacture of calcium gluconate and similar products.....	1146
III. Experimental details.....	1147
1. Apparatus and method used.....	1147
2. Oxidations of <i>d</i> -glucose, <i>d</i> -galactose, and <i>d</i> -mannose.....	1148
3. Oxidations of <i>d</i> -xylose, <i>l</i> -arabinose, and <i>l</i> -rhamnose.....	1150
4. Oxidations of <i>d</i> -lactose and <i>d</i> -maltose.....	1151
IV. Acknowledgment.....	1152

## I. INTRODUCTION

The widespread interest in the use of calcium gluconate for the medical treatment of calcium deficiency makes the discovery of an improved method for its preparation of unusual importance. The study of the oxidation of aldose sugars in the presence of various buffers was begun by C. S. Hudson and the senior author several years ago. The early work was largely confined to the study of bromine oxidation in the presence of different buffers. A very convenient method for the laboratory preparation of the monocarboxylic sugar acids was developed.<sup>1</sup> However, this method, as well as the other methods heretofore used, is not wholly satisfactory for the commercial manufacture of sugar acids or their salts. For if oxidation is effected by bromine<sup>2</sup> or by chlorine<sup>3</sup> two molecules of hydrogen

<sup>1</sup> Hudson and Isbell, B. S. Jour. Research, **3**, p. 57, (RP82); 1929; also J. Am. Chem. Soc., **51**, p. 2225; 1929.

<sup>2</sup> Kiliani and Kleemann, Ber., **17**, p. 1296; 1884.

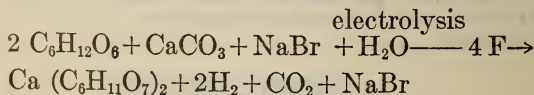
<sup>3</sup> Stoll and Kussmaul, U. S. Patent 1648368; 1927.

bromide or hydrogen chloride are formed for each molecule of sugar which is converted to the acid. This large amount of hydrogen bromide decreases the reaction rate. This retarding effect may be eliminated by the addition of a base or buffer, such as calcium carbonate,<sup>4</sup> but even then a large quantity of calcium bromide is formed, which seriously interferes with the separation of the desired product. The fermentation method<sup>5</sup> for the preparation of calcium gluconate does not have the objectionable features just mentioned, but it does not give a quantitative yield, and requires careful control lest foreign organisms enter the vats and produce undesirable side reactions.

Electrolytic oxidations are not only cheap and convenient, but they have, in addition, the decided advantage that no undesirable substances are produced from the oxidant. By the usual electrolytic oxidation, the aldose sugars are not oxidized in appreciable quantity to the corresponding aldonic acids. Thus, Löb<sup>6</sup> found that glucose on electrolytic oxidation gives a whole series of degradation products, including *d*-arabinose and formaldehyde.

## II. A NEW PROCESS FOR THE MANUFACTURE OF CALCIUM GLUCONATE AND SIMILAR PRODUCTS

The undesirable features of the older methods for the preparation of sugar acids have been overcome by a new process wherein oxidation is effected electrolytically in the presence of a small quantity of a bromide. As a result, a nearly quantitative oxidation of the sugar into its monocarboxylic acid is obtained without objectionable by-products. In the preparation of calcium salts the oxidation is conveniently conducted in the presence of the bromide and calcium carbonate. The calcium carbonate reacts with the sugar acid as fast as it is formed and thus maintains a nearly neutral solution in which the bromine is absorbed as fast as it is liberated by electrolysis. The total reaction is represented by the following equation:



Many of the calcium salts of the sugar acids crystallize very readily from water solution. Thus, when a concentrated solution of glucose is oxidized in the manner outlined, calcium gluconate crystallizes from the solution toward the end of the reaction. This may be separated by filtration, after which additional glucose and calcium carbonate may be added to the mother liquor and the process repeated. The bromide is thus recovered in the mother liquors; hence a small amount of it will effect the oxidation of an indefinitely large amount of sugar. The only bromide which is lost is that which contaminates the product and even this bromide may be largely recovered from the mother liquors from a second crystallization. In addition to expediting the desired reaction the bromide causes an increase in the conductivity of the solution, thus reducing the resistance and hence the amount of energy wasted as heat. The quantity of bromide used in

<sup>4</sup> Clowes and Tollens, *Ann.*, **310**, p. 166; 1900.

<sup>5</sup> May, Herrick, Moyer, and Hellbach, *Ind. Eng. Chem.*, **21**, p. 1198; 1929.

<sup>6</sup> Löb, *Biochem. Z.*, **17**, pp. 132, 243; 1909.



the experimental work reported in this publication is more than the minimum amount required for a smooth reaction. The optimum quantity for use in the commercial preparation of these derivatives depends on many factors, including the economic aspects of the subject which will not be considered in this publication.

The oxidation of 1 mol of an aldose sugar to the corresponding acid requires 1 atom of oxygen or 2 Faradays. The actual quantity of electricity required for the oxidations is very near this amount. Thus 45 g (0.25 mol) of glucose is oxidized to the extent of 95 per cent by 0.5 Faraday (13.4 ampere-hours). The fact that two equivalents of electricity cause the disappearance of 95 per cent of the reducing power of the sugar (Scales method) shows that the principal reaction is the oxidation of the sugar to the corresponding acid, because any other oxidation reaction which would decrease the reducing power of the solution to the same extent would require several equivalents of electricity.

The high current efficiency, together with the low potential required, makes the process very economical. The cost for the electricity used in the oxidation is extremely small as compared with the cost of bromine or even chlorine as an oxidizing agent. The yield of the final product is nearly quantitative; in fact, as high a yield may be obtained as may be obtained by the use of expensive reagents.

### III. EXPERIMENTAL DETAILS

#### 1. APPARATUS AND METHOD USED

A very simple electrolytic cell was used. This consisted of two electrodes fitted in a 2-liter three necks Pyrex distilling flask which was equipped with a mechanical stirrer. The electrodes consisted of round graphite rods 22 mm in diameter and sufficiently long to reach the bottom of the flask. They were placed in the outer necks of the flask as illustrated in Figure 1, while the stirrer was inserted in the center. The sugar solution containing the bromide, and calcium carbonate in suspension, was placed in the flask and a direct current was passed between the electrodes. A potential of 15 volts gave a current of slightly more than 0.5 ampere, or a current density of 10 amperes per square decimeter. The potential is considerably more than that required for the actual oxidation, the loss being largely due to the resistance of the electrodes and of the solution. The quantity of electricity was measured by a copper coulometer in series with the oxidation cell. As the reaction proceeded, hydrogen and carbon dioxide escaped from the flask. The reaction mixture remained only slightly acid, and colorless, the bromine being absorbed as fast as it was liberated. Samples were taken at intervals and the reducing sugar was determined by copper reduction or iodine titration. The samples were also tested for the presence of free bromine with potassium iodide and starch. In all cases negative tests for free bromine were obtained until the reaction was nearly complete, as indicated by only a faint Fehling's test for reducing sugar. The completion of the reaction could be detected directly by the appearance of a faint yellow coloration which occurred when slightly more than the theoretical quantity of current had been used. At the end of the reaction the sugar was nearly completely converted into the calcium salt of the

corresponding monobasic acid. The previously known calcium salts of gluconic,<sup>7</sup> mannonic,<sup>8</sup> galactonic,<sup>9</sup> arabonic,<sup>10</sup> and rhammonic<sup>11</sup> acids were prepared by simple evaporation of the solutions and crystallization of the products. The mother liquors may be used in subsequent oxidations, thus giving a very efficient method for the conversion of the sugars into salts of the sugar acids. Xylonic, maltobionic, and

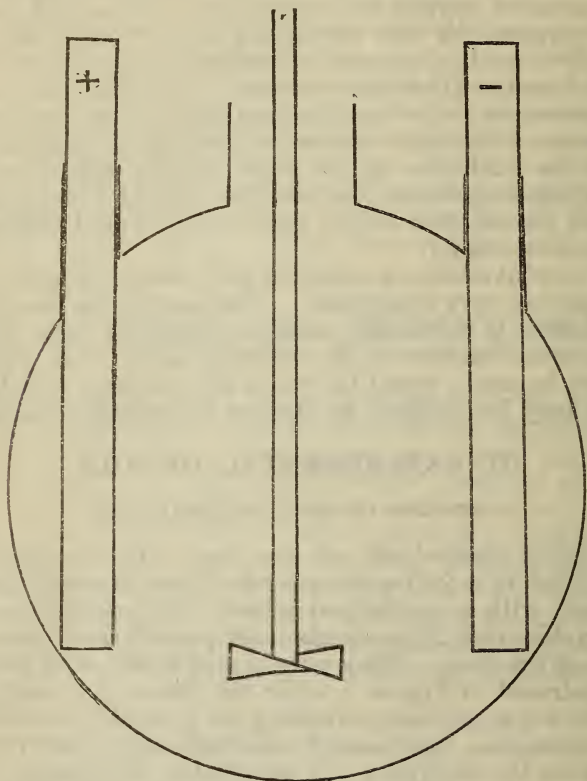


FIGURE 1.—Oxidation cell

lactobionic acids were separated by the formation of insoluble basic calcium salts.<sup>12</sup> These salts may be used for the preparation of any desired derivative.

## 2. OXIDATIONS OF *d*-GLUCOSE, *d*-GALACTOSE, AND *d*-MANNOSE

A solution consisting of 45 g of anhydrous *d*-glucose (0.25 mol) and 8 g of calcium bromide (anhydrous) in sufficient water to give 1 liter was placed with 25 g of calcium carbonate in the apparatus illustrated in Figure 1. A direct current of 0.5 ampere was passed through the solution and the quantity of electricity was measured with a copper coulometer. Samples of the solution were taken from

<sup>7</sup> Fischer, Ber., 23, p. 2614; 1890.

<sup>8</sup> Fischer and Hirschberger, Ber., 22, p. 3220; 1889.

<sup>9</sup> Schnelle and Tollens, Ann., 271, p. 81; 1892.

<sup>10</sup> Killiani, Ber., 19, p. 3029; 1886.

<sup>11</sup> Rayman, Ber., 21, p. 2046; 1888.

<sup>12</sup> Hudson and Isbell, B. S. Jour. Research, 3 (RP82), p. 60; 1929.

time to time, and the amount of sugar remaining in the solution was determined by Scales<sup>13</sup> method. The difference between the amount of sugar at the beginning of the reaction and at any given time is equal to the amount of sugar oxidized. The results are shown in Figure 2. The amount of oxidation is nearly proportional to the quantity of current used, and the oxidation is virtually complete when the theoretical quantity (13.4 ampere-hours) of electricity has passed through the solution. Qualitative tests for free bromine on small portions of the solution were negative until slightly more than the theoretical amount of current had been used, after which positive tests were obtained. The tests were made with potassium iodide and starch solution in the usual manner. An excess of bromine is slowly absorbed by the calcium salts of sugar acids, and hence the solutions

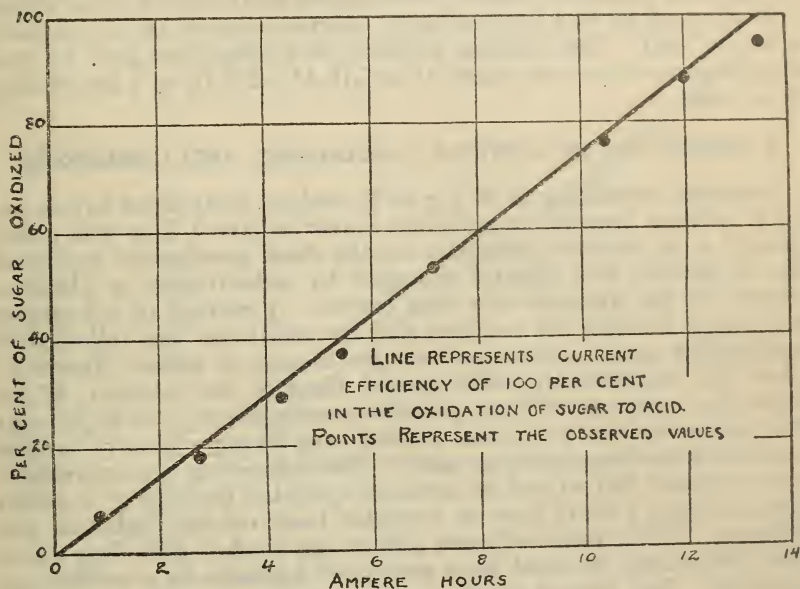


FIGURE 2.—Oxidation of glucose

were not allowed to stand more than two or three minutes before making the tests.

The current was interrupted when the sugar determination showed that 96 per cent of the sugar had been oxidized to the monobasic acid. The solution was filtered and then concentrated in vacuo to a thin sirup from which crystalline calcium gluconate was readily separated. A total of 45 g of the salt,  $\text{Ca}(\text{C}_6\text{H}_{11}\text{O}_7)_2 \cdot 2\text{H}_2\text{O}$  was obtained, representing a yield of about 77 per cent of the theoretical. The calcium bromide in the mother liquors prevents the complete crystallization of the calcium gluconate, but the residual salt may be recovered in subsequent runs if additional sugar is added and the process repeated. However, if it is desired to recover the maximum quantity of calcium gluconate the calcium bromide may be removed with silver oxalate.<sup>14</sup>

<sup>13</sup> Scales, *J. Ind. Eng. Chem.*, **11**, p. 747; 1919.

<sup>14</sup> Isbell, *B. S. Jour. Research*, **5** (RP226), p. 753; 1930.



The solution thus freed from the calcium bromide gives additional calcium gluconate on evaporation.

The oxidation of galactose and of mannose was conducted in a manner analogous to that just outlined for glucose. The results were similar in that the sugars were virtually quantitatively converted into calcium salts of the corresponding sugar acids.

Forty-five grams (0.25 mol) of *d*-galactose was oxidized to the extent of 98 per cent by 13.3 ampere-hours representing an anode efficiency of 99 per cent. The reaction product on evaporation gave 35 g of crystalline calcium galactonate,  $(\text{Ca}(\text{C}_6\text{H}_{11}\text{O}_7)_2 \cdot 5\text{H}_2\text{O})$  which represents a net yield of 54 per cent. The residual calcium galactonate could be obtained only after removing the calcium bromide from the mother liquors.

Forty-five grams (0.25 mol) of *d*-mannose was oxidized to the extent of 94 per cent by 13.4 ampere-hours, representing an anode efficiency of 94 per cent. The reaction product on evaporation gave 40 g. of crystalline calcium mannonate,  $(\text{Ca}(\text{C}_6\text{H}_{11}\text{O}_7)_2 \cdot 2\text{H}_2\text{O})$  or a net yield of 69 per cent.

### 3. OXIDATIONS OF *d*-XYLOSE, *l*-ARABINOSE, AND *l*-RHAMNOSE

A solution consisting of 37.5 g (0.25 mol) of anhydrous xylose and 8 g of calcium bromide in sufficient water to give 1 liter was placed with 25 g of calcium carbonate in the flask previously described. The apparatus was slightly changed by substituting a platinum cathode for the graphite one used before. A current of 0.4 ampere was passed through the solution and the oxidation was followed by quantitative sugar determinations by means of iodine titration.<sup>15</sup> After 13.9 ampere-hours were passed through the solution, 97 per cent of the sugar was oxidized, giving an anode efficiency of 93 per cent. The calcium xylonate which is formed may be conveniently isolated by use of a new basic calcium salt.<sup>16</sup> The solution is concentrated in vacuo to about 200 ml and an excess of hydrated lime (25 g) is added. After standing a short time an insoluble basic calcium xylonate precipitates. This is collected upon a filter and washed with lime water. The filtrate may be used as a source of bromide in a subsequent oxidation. The normal calcium xylonate may be made from the basic salt by removing the excess of lime with carbon dioxide. After filtration and evaporation of the aqueous solution a thick sirup is obtained which may be reduced to an amorphous powder by drying.

The following experiment shows that as the oxidation of the sugar progresses more sugar may be added to replace that which is converted to the calcium salt, and thus build up the concentration of the desired product. In the case of xylose the calcium xylonate is very soluble, and hence it does not crystallize from the solution. But with glucose, crystalline calcium gluconate forms which may be separated by filtration from time to time and the process continued.

Fifty-seven grams of xylose and 15 g of sodium bromide dissolved in 1,500 ml of water were placed in the oxidation flask with 37 g of calcium carbonate. Electrolytic oxidation was conducted with a current of 0.3 ampere. At intervals of 21 ampere-hours four additional portions of xylose (57 g each) and calcium carbonate (20 g each)

<sup>15</sup> Kline and Acree, B. S. Jour. Research, 5 (RP247), p. 1063; 1930.

<sup>16</sup> The composition of this salt, as well as the composition of the corresponding salts of maltose and lactose, is being investigated.

were added. After 109 ampere-hours oxidation was nearly complete, only 3 per cent of the 285 g of xylose remained in the solution, representing an anode efficiency of 91 per cent.

The oxidation of *l*-arabinose and of *l*-rhamnose was conducted in a slightly smaller apparatus than that employed in the experiments on glucose and galactose, but essentially the same conditions were maintained except that both electrodes were of platinum instead of graphite. The quantities of the reagents were one-half those used in the first xylose experiment.

An 18.75 g (0.125 mol) sample of *l*-arabinose was oxidized to the extent of 91 per cent by 8.0 ampere-hours of current. The reaction product on evaporation gave 14 g, or a net yield of 49 per cent, crystalline calcium arabonate ( $\text{Ca}(\text{C}_5\text{H}_9\text{O}_6)_2 \cdot 5\text{H}_2\text{O}$ ). The anode efficiency (76 per cent) was not as high as that obtained with some of

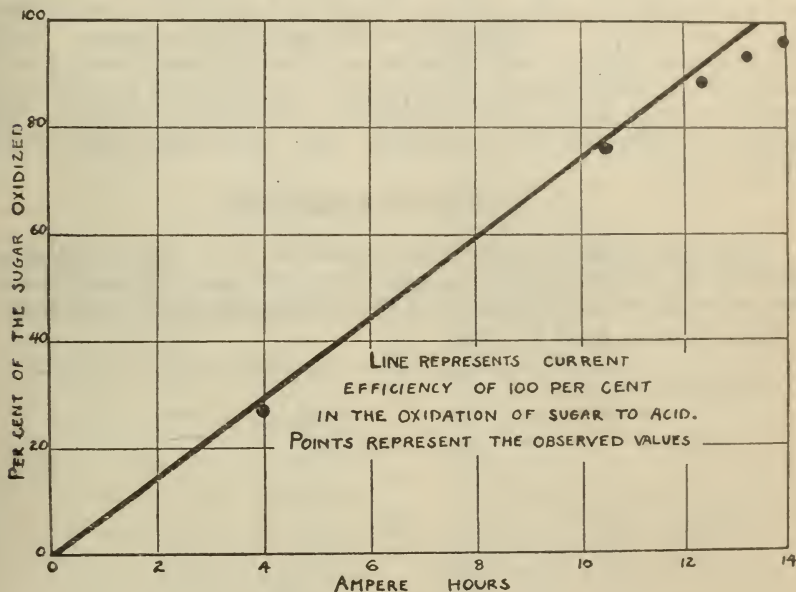


FIGURE 3.—Oxidation of lactose

the other sugars. This was presumably due to the formation of an insoluble crust on the cathode which appears to be a basic calcium arabonate. This subject will be considered in a future publication.

An 18.75 g (0.103 mol) sample of *l*-rhamnose (monohydrate) was oxidized to the extent of 94 per cent by 6.4 ampere-hours, representing an anode efficiency of 81 per cent. The reaction product, on evaporation, gave 19 g of crude crystalline calcium rhamnonate.

#### 4. OXIDATIONS OF *d*-LACTOSE AND *d*-MALTOSE

A solution consisting of 90 g (0.25 mol) of *d*-lactose (monohydrate) and 8 g of calcium bromide in sufficient water to give 1 liter was placed, together with 25 g of calcium carbonate, in the apparatus used for the oxidation of xylose. A current of 0.4 ampere was passed through the solution and the oxidation was followed by iodine titration of the unoxidized sugar. The results, shown in Figure 3, clearly

indicate that the reaction proceeded with the same regularity which characterized the oxidations of the monosaccharides. The use of calcium carbonate in the oxidation of the reducing disaccharides maintains a nearly neutral solution so that little or no hydrolysis of the disaccharides occurs. After the theoretical quantity of current had been consumed the reaction was about 95 per cent complete. The solution was treated with lime (25 g) and the product was obtained as the basic calcium lactobionate previously reported by C. S. Hudson<sup>17</sup> and the senior author.

The oxidation of *d*-maltose (monohydrate) was conducted in manner analogous to that given for lactose. After 13.9 ampere-hours, sugar determinations showed that 98 per cent of the sugar had been oxidized, with an anode efficiency of 94 per cent. The product was isolated by means of a new basic calcium maltobionate. The solution was concentrated to about 300 ml and an excess of hydrated lime (25 g) was added. A difficultly soluble precipitate formed. After standing overnight it was collected upon a filter and washed with lime water. The normal calcium salt was prepared in analogous manner to that used for the preparation of calcium xylionate. The product so obtained was amorphous and, when thoroughly dried, weighed 60 g.

#### IV. ACKNOWLEDGMENT

The authors express their appreciation to F. J. Bates and other members of the polarimetry section who have been of assistance in the course of the investigation and the preparation of the manuscript.

WASHINGTON, April 27, 1931.

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<sup>17</sup> See footnote 1, p. 1145.