

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF MARQUETTE UNIVERSITY]

The Electrolytic Preparation of Glucuronic Acid

BY R. A. LEUTGOEB¹ AND HERBERT HEINRICH

Glucuronic acid is generally prepared by feeding to dogs phenols and certain toxic substances which combine in the body with glucose and are eliminated as conjugation products of glucuronic acid.² Another promising biological method is the preparation by the hydrolysis of gum arabic.³ The strictly chemical methods are based on either the reduction of saccharic acid⁴ or the oxidation of glucose.⁵ Recently, Isbell, Frush and Bates⁶ have worked out a method to prepare gluconic acid by electrolytic oxidation of glucose. Such an oxidation offers a number of advantages which make it seem worth while to adapt it to the preparation of glucuronic acid.

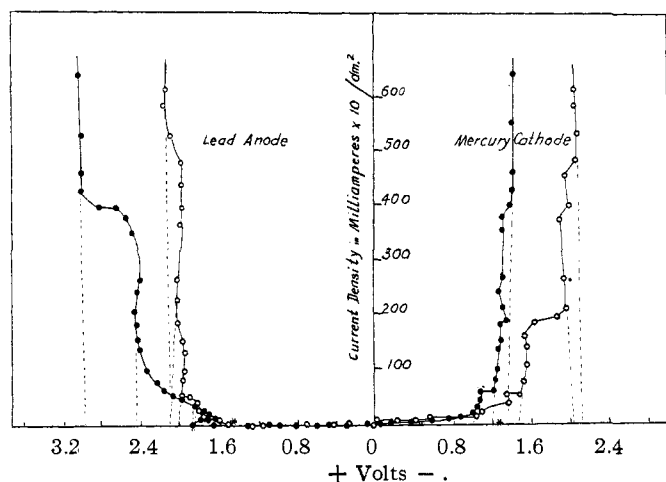


Fig. 1.—Decomposition potentials of the mixture sulfuric acid, sodium sulfate, methyl glucoside, and atmospheric oxygen: ●, α -methyl glucoside; ○, β -methyl glucoside; *, breaks of inorganic ions.

The simple anodic oxidation cannot be employed because the carbonyl group has to be protected by some group usually in the form of a glucoside which, however, is readily hydrolyzed by the acid liberated at the anode during elec-

(1) Portion of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in chemistry, 1938.

(2) Schmiedeberg and Mayer, *Z. physiol. Chem.*, **3**, 422 (1879); Ehrlich and Rehorst, *Ber.*, **58**, 1989 (1925); A. Quick, *Ind. Eng. Chem., Anal. Ed.*, **5**, 26 (1933); Z. S. Kertesz, *J. Biol. Chem.*, **108**, 127 (1935).

(3) F. Weinmann, *Ber.*, **62B**, 255 (1929).

(4) E. Fischer and O. Piloty, *ibid.*, **24**, 521 (1891).

(5) A. Jolles, *Chem. Z.*, **34**, 242 (1911); M. Bergmann and W. Wolff, *Ber.*, **56B**, 1060 (1923).

(6) Isbell, Frush and Bates, *J. Ind. Eng. Chem.*, **24**, 375 (1932).

trolysis. Therefore, it was decided to use cathodic oxidation as described by Traube.⁷ In this process oxidation occurs at the cathode through reduction of gaseous oxygen by electrolytic hydrogen to hydrogen peroxide. The original method recommends amalgamated copper cathodes, but extensive experiments proved that it is impossible to prevent copper from dissolving in the electrolyte, and it was therefore decided to use pure mercury cathodes, and to bubble air or oxygen through the liquid metal. Lead anodes are covered with several layers of stout canvas to reduce diffusion of the acid from the anode. The electrolyte consists of sodium sulfate, a trace of sulfuric acid and methyl glucoside prepared by the method of Patterson and Robertson.⁸

In order to establish the potential at which methyl glucoside is oxidized to the corresponding uronic acid, the decomposition potential curves of the electrolyte were determined (Fig. 1). By independent measurements made on solutions containing only sodium sulfate and sulfuric acid, those breaks which were caused by the inorganic ions, were found and are indicated in Fig. 1. The remaining breaks were investigated by electrolyzing at each critical voltage for several hours and testing the electrolyte for glucuronic acid by means of the naphthorescincinol reagent.⁹

This investigation proved that glucuronic acid is produced at the lowest cathode decomposition potential. This fact eliminates as impurities all substances except those present in the original electrolyte. It has, however, the serious disadvantage of extremely low permissible current densities. Since using an enlarged cathode surface did not increase the yield sufficiently, stirring had to be resorted to. A comparison of the graphs on Figs. 1 and 2 indicates that stirring does allow the preparation of glucuronic acid at higher current densities.

Stirring improves the permissible cathode current density apparently because it distributes the hydrogen peroxide throughout the electrolyte and

(7) Traube, *Ber.*, **15**, 2434 (1882).

(8) Patterson and Robertson, *J. Chem. Soc.*, 300 (1929).

(9) C. Tollens, *Ber.*, **41**, 1788 (1908).

lowers the polarization. Decreasing the oxygen pressure will also lower the concentration of hydrogen by the ready formation of hydrogen peroxide, which in turn is rapidly used up in the oxidation of methyl glucoside.

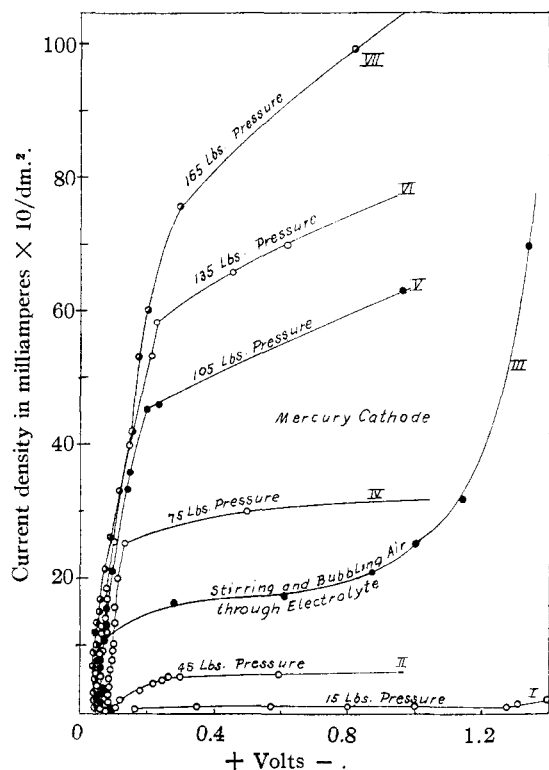


Fig. 2.—Decomposition potentials of α -methyl glucoside.

The decomposition potential curves (Fig. 2) at pressures up to 11 atm. per sq. in. were determined while the electrolytic cell and the calomel cell were enclosed in a small steel shell. Increasing the pressure caused the permissible current density to rise from 0.03 amp. at atm. pressure to 1 amp. per sq. dm. at a pressure of 11 atm.

Table I supports the contention that stirring of the electrolyte is equivalent to a certain amount of oxygen pressure and that, over the pressure range investigated, stirring helps the attaining of the equilibrium voltage which seems to be about 0.06 v. compared to the 1 *N* calomel cell as zero.

TABLE I

INFLUENCE OF PRESSURE AND AGITATION ON THE POTENTIAL DIFFERENCE BETWEEN THE MERCURY CATHODE AND THE 1 *N* CALOMEL CELL

Pres. of oxygen, lb.	15	45	75	105	135	165
Potential of cathode without stirring, v.	0.474	0.418	0.330	0.104	0.090	0.086
Potential of cathode with stirring, v.	.474	.400	.340	.120	.095	.088
Potential of cathode without stirring, v.	.172	.115	.040	.068	.054	.060
Potential of cathode with stirring, v.	.180	.100	.088	.080	.060	.062

On the basis of the decomposition potentials, methyl glucoside was oxidized at 11 atm. at a current density of 0.3–0.6 amp. per sq. dm. The oxidation was controlled by keeping the voltage difference between the 1 *N* calomel cell and the mercury cathode below 0.3 v., *i. e.*, below the next higher decomposition potential. The results of three runs, tabulated in Table II, seem to indi-

TABLE II

THE ELECTROLYTIC PREPARATION OF METHYL GLUCURONIDE FROM METHYL GLUCOSIDE

No. of run	1	2	3
Methyl glucoside used, g.	97	48.5	97
Total loss of pressure in steel shell, lb.	23	17	32
Approx. vol. of tank, l.	7.5	8.0	7.5
Approx. amt. of O ₂ absorbed, g.	15.3	9.4	22.0
Total cathode surface, sq. dm.	1.74	0.87	1.74
Av. current, amp.	1.0	.433	0.62
Time of electrolysis, hrs.	54	86	85
Theoretical time of reaction at 100% current efficiency, hrs.	53.6	61.8	86.4
BaCO ₃ obtained, g.	4	6	2.8
CO ₂ formed in electrolysis, g.	0.8	1.2	0.56

cate that it is more advantageous to electrolyze at lower current densities for a longer time because more oxygen is absorbed, less carbon dioxide liberated, and by actual test more glucuronic acid formed. The carbon dioxide is probably an oxidation product of uronic acid. The amount of oxygen was estimated from the pressure drop and was replaced several times during electrolysis.

The separation of methyl glucuronide from the electrolyte and the isolation of crystalline glucuronic acid are difficult and require further investigation. The customary precipitation method using barium or lead glucuronates as intermediates yielded in some cases as much as 30 g. of salt which on addition of sulfuric acid or hydrogen sulfide, respectively, yielded a sirup which gave a strong test for glucuronic acid and which, by extraction with alcohol, gave a small quantity of crystalline glucuronic lactone. Crystalline sodium glucuronate was prepared from the sirup but was found to be impure and not suitable for the isolation of the product. The most promising intermediate for preparing the product appears to be the cinchonine derivative of glucuronic acid, of which 2.8 g. was prepared from 6 g. of methyl glucoside, indicating 20.2% conversion in terms of methyl glucoside taken. The constants of the derivative were m. p. 198.5–199°, $[\alpha]^{20}_D +138^\circ$.

Experimental

Determination of Decomposition Potential Curves.—The electrolyte consisted of 0.7 cc. of concd. sulfuric acid, 0.1 mole of glucose or α - or β -methyl glucoside dissolved in enough of 0.1 *N* sodium sulfate solution to give 100 cc. of solution. The decomposition potential curves were obtained by the method of Le Blanc¹⁰ in which the potential difference between a reference electrode and the electrode is measured by means of the Poggendorff compensation method, while increasing voltages are impressed on the system. The decomposition potential curves of sodium sulfate, sulfuric acid and glucose indicated those breaks which are caused by the discharge of ions from any of these three compounds. Tests at each of the remaining breaks proved that the formation of glucuronic acid occurs only at the lowest decomposition potential of about -0.06 to -0.16 v. compared to the 1 *N* calomel cell. The tests for glucuronic acid are in general based on Tollens⁹ naphthoresorcinol test, of which several modifications¹¹ have been proposed to make it more specific for glucuronic acid. Whenever glucuronic acid occurred in the electrolyte the various tests were strongly positive.

Electrolysis at Higher Pressures.—The pressure vessel, 59 cm. long and 14 cm. in diameter, is a steel shell connected to an oxygen tank, and is fitted with pressure gage and safety valve. A thick rubber gasket between the pressure vessel and a heavy steel plate assures airtight seal. The steel shell, fastened to a wooden carriage resting on four roller-coasters, is moved forward and backward at a rate of 45 shifts a minute by a $1\frac{1}{4}$ horsepower motor, operating an eccentric by means of a worm gear. This rate and kind of motion were found to be most effective in mixing the electrolyte by producing an oscillating horizontal wave in the mercury cathode.

The current in the applied voltage circuit, taken from a 20-volt d. c. generator, is measured by a milliammeter and adjusted by means of a slide-wire rheostat. The voltage difference existing within the pressure vessel between the 1 *N* calomel cell and the mercury cathode is measured by Poggendorff's compensation method.

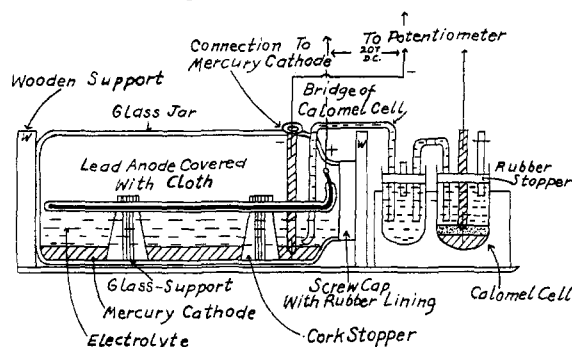


Fig. 3.—Electrolytic cell.

The electrolytic apparatus (Fig. 3) is constructed of two one-liter Mason jars fitted into the steel shell which permit experiment with half quantities. Holes are drilled

(10) Le Blanc, *Z. physik. Chem.*, **8**, 299 (1891).

(11) Neuberger and Saneyoski, *Biochem. Z.*, **36**, 56 (1911); Tollens and Stern, *Ber.*, **41**, 1788 (1908); Wheeler and Tollens, *Ann.*, **254**, 333 (1889); Mylius, *Z. physiol. Chem.*, **11**, 494 (1887); Goldschmidt, *ibid.*, **65**, 390 (1910).

through the walls of the jars for the introduction of various connections to the electrodes. The lead anode, sewed in several layers of cloth, is raised above the mercury cathode by means of small glass supports passing through corks. This arrangement prevents contamination of the electrolyte by copper and iron which, as experiments proved, corrode rapidly in the electrolyte under the influence of compressed oxygen. The mercury cathodes, connected to each other and the outside circuit by means of platinum wires sealed into glass tubes filled with mercury, have a combined surface of 1.74 sq. dm. The 1 *N* calomel cell is connected through an intermediate vessel with the electrolytic cell. Both, calomel cell and intermediate vessel, are set into a block of wood, and the intermediate vessel is fitted with a small open tube to allow pressure equalization.

The Isolation of Glucuronic Acid.—The electrolyte is hydrolyzed by refluxing the solution gently for three hours after adding enough sulfuric acid to it to make the concentration 0.2 *N* acid. Precipitation of the liberated glucuronic acid by means of basic lead acetate yielded a precipitate which on addition of hydrogen sulfide, followed by filtration, concentration, and repeated extraction with absolute alcohol, yielded only a small amount of crystalline lactone of m. p. 170°, the recorded one being 170°. A little larger amount of crystalline lactone having the proper m. p. and the characteristic bitter-sweet taste could be obtained by Weinmann's method¹³ which makes use of the barium salt of which about 30 g. was obtained from 250 cc. of electrolyte.

The most promising intermediate suitable for the recovery of glucuronic acid, seems to be cinchonine glucuronate first described by Carl Neuberger.¹⁴ For the preparation of this compound, the free sulfuric acid in the hydrolyzed electrolyte was neutralized by barium hydroxide, the precipitated barium sulfate filtered off, and the filtrate concentrated at room temperature by means of a high vacuum. The sirup was extracted with hot alcohol and, after evaporation of the alcohol, the resulting residue taken up in water. One sixteenth of the total aqueous solution, equivalent to about 6 g. of methyl glucoside, was diluted with water and purified by charcoal. While the purified solution was boiling, cinchonine in small portions at a time was added until no more of it dissolved; any excess of cinchonine could be removed by extraction with ethyl acetate followed by chloroform. The solution, again purified by charcoal, was kept on a water-bath until needle-like crystals of cinchonine glucuronate crystallized out. They were redissolved in absolute alcohol, the solution filtered, and the cinchonine glucuronate again crystallized by the addition of water, followed by a concentration of the aqueous alcoholic solution.

The yield was 2.8 g., or 20.2% in terms of methyl glucoside taken. After three recrystallizations and purifications with charcoal, the specific rotation of a 2% solution was $[\alpha]_{20}^D +138^\circ$, and the m. p. 189.5–199°. Neuberger¹⁴ gives the m. p. of cinchonine glucuronate as 204°, and $[\alpha]_{20}^D +138.6^\circ$. Ehrlich and Rehorst¹⁵ claim that the m. p. is 199–200°.

(12) Mann and Tollens, *Ann.*, **290**, 155 (1896).

(13) F. Weinmann, *Ber.*, **62B**, 255 (1929).

(14) C. Neuberger, *ibid.*, **33**, 3315 (1900).

(15) Ehrlich and Rehorst, *ibid.*, **58**, 1989 (1925).

Summary

1. Methyl glucoside can be oxidized cathodically to methyl glucuronide. This electrolytic oxidation proceeds best at higher pressures.

2. At pressures of 11 atm., with agitation and at room temperature, over 20% conversion of methyl glucoside into glucuronic acid could be obtained.

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The Densities of Vapors Adsorbed on Charcoal

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Only two direct measurements of the density of adsorbed gases or vapors are available in literature. Ewing and Spurway² determined the density of water on silica gel at 25°. De Vries³ determined the density of carbon dioxide on charcoal at 30° and showed that the density of the adsorbed layer could be calculated from an adsorption isotherm, using the Lowry and Olmstead⁴ modification of the Polanyi theory. In neither of these two cases were the densities determined over the entire isotherm of the adsorbed substance. Emmett and Brunauer⁵ calculated, from an adsorption isotherm, the volume of gas needed to form a monomolecular layer and from that the surface area of the adsorbent, assuming that the adsorbed layer had a density comparable to that of the normal liquid. In our work the densities of carbon tetrachloride and acetone, adsorbed on charcoal, were determined at 30° over the entire isotherm up to near saturation pressures. The results verify the choice of density made by Emmett and Brunauer.

Experimental and Results

Procedure.—A commercially available steam-carbon dioxide activated coconut charcoal was used. For the carbon tetrachloride and the acetone experiments, 11.88 and 10.23 g. of charcoal were used, respectively.

Dow Chemical Co. carbon tetrachloride of 99.9% purity and Mallinckrodt analytical grade of acetone were used.

The apparatus and experimental method was essentially similar to that described by De Vries.³ The charcoal was degassed under high vacuum at 375° for ten to fifteen hours. All pressure measurements were made in triplicate by slightly changing the height of the mercury column and then making the corrections described in the above-

mentioned paper. The final data are good to about 3% for the low pressure values and to about 1% for the higher pressures. The volume of the free space in the tubes containing the adsorbent was determined with carefully purified helium. For the carbon tetrachloride experiments the mean value of nine determinations scattered amongst the density measurements was 21.840 ± 0.008 ml. For the acetone experiments the volume of the free space in the bulb was similarly found to be 23.077 ± 0.035 ml.

A known amount of the organic liquid, previously made air-free, was allowed to vaporize into the charcoal bulb, and after five to eight hours, when the pressure became steady or changed very slowly at a known rate, a measured amount of helium was introduced. The total pressure was measured after one to three hours, a correction being made for the partial pressure of the organic vapor. When larger amounts of material were adsorbed, the pressure did not approach a steady value so quickly. The partial pressure of the vapor was then calculated, using the three constant equation, $p = a + bt^n$, for the extrapolation.

In this work the assumption has been made that helium is not adsorbed on the charcoal at 30°, and that it does not influence the amount of vapor adsorbed after it is added to the vapor already in equilibrium with the adsorbent. Homfray⁶ has reported that helium was not measurably adsorbed on an active charcoal at room temperature under a pressure of 70 cm. One of us determined on a previous occasion³ the rate at which helium was absorbed by 17.5 g. of coconut charcoal. The unpublished result of numerous experiments, some extending over as much as sixty hours with helium at a pressure of 55 cm., was that helium was "dissolving" in the charcoal at a uniform rate of 0.00082 ml. per hour. We therefore believe that in our experiments when measurements were taken after one to three hours, and when no appreciable change in total pressure was observed after that time, no more than what could be accounted for by the slow change in the equilibrium pressure of the organic vapor on the charcoal, the volume of the free space could be calculated after subtracting the partial pressure of the vapor from the total pressure of the helium plus the vapor. Furthermore, Lambert and Heaven⁷ have reported that helium is not adsorbed on silica gel at 0°, and that oxygen is adsorbed from an O₂-He mixture in the same amount that it would be in the ab-

(1) Present address: Universal Oil Products Co., Riverside, Ill. This paper is an abstract of the thesis submitted by J. D. Danforth in partial fulfillment of the requirements for the degree of Doctor of Philosophy in chemistry.

(2) Ewing and Spurway, *THIS JOURNAL*, **52**, 4635 (1930).

(3) De Vries, *ibid.*, **57**, 1771 (1935).

(4) Lowry and Olmstead, *J. Phys. Chem.*, **31**, 1601 (1927).

(5) Emmett and Brunauer, *THIS JOURNAL*, **69**, 1553 (1937); Brunauer, Emmett and Teller, *ibid.*, **60**, 309 (1938).

(6) Homfray, *Z. physik. Chem.*, **74**, 129 (1910).

(7) Lambert and Heaven, *Proc. Roy. Soc. (London)*, **A153**, 584 (1936).