DERIVATIVES OF GLUCURONIC ACID

I. THE PREPARATION OF GLUCURONIC ACID FROM GLUCURON AND A COMPARISON OF THEIR REDUCING VALUES

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The presence of glucuronic acid in the complex molecules of specific bacterial polysaccharides (1) has stimulated a study of certain derivatives of this important substance and its lactone, glucuron. These investigations have entailed the necessity of elaborating a satisfactory method for preparing both the acid and its lactone, and at the same time they have embraced a study of the comparative reducing values of these two substances. The present communication presents the results of this work.

Preparation of Source Material—A consideration of the various available sources and methods for the preparation of glucuronic acid has led us to the adoption of the excellent method of Quick (2) for securing large quantities of this compound in the form of the zinc salt of the borneolglycoside of glucuronic acid. Although the method of Weinmann (3), who prepared glucuronic acid from an acidic gum (4) derived from gum arabic, as well as the method of Fischer (5), offers available sources of the material, both of these methods have certain drawbacks. The method of Quick, on the other hand, proved to us to be of utmost value, for not only is it simple, but it provides a source material of high purity without elaborate chemical procedure.

Thus, eight dogs weighing 7 to 12 kilos each were housed in clean metabolism cages. They were fed a daily diet of ½ pound of ground raw horse meat mixed with a little bread, salt, and 7 gm. of pulverized β-borneol, during 4 consecutive days. During the succeeding 3 days of the week, the animals were maintained on a borneol-free diet. When these conditions were followed the
general health of the dogs remained excellent, and the ultimate output of conjugated glucuronic acid was greater than that obtained when the animals were fed on a continuous diet of borneol. The urine of the animals was collected during the first 4 days. The conjugated borneolglycoside was isolated from the urine, according to Quick, as its zinc salt.\(^1\)

Borneylglucuronide was also obtained as described by Quick, and the hydrolysis of the glycoside with 0.2 \(\text{N} \ H_2\text{SO}_4\) was carried out according to his directions. After the hydrolysis, however, we found it advisable to remove the sulfuric acid with barium carbonate rather than with barium hydroxide, because glucuronic acid is extremely sensitive to alkali. After removing the sulfuric acid, the aqueous solution of glucuronic acid and its lactone was evaporated \emph{in vacuo} to a thick syrup. It was then dissolved in absolute alcohol, and reevaporated, the process being repeated three times in all. A beautiful crystalline product, consisting of a mixture of free glucuronic acid and of its lactone, was thus obtained.

We cannot recommend too highly Quick's method for the preparation of glucuronic acid-lactone mixture. The yield of the conjugated zinc salt, from eight dogs, varied from 100 to 160 gm. per 4 days of feeding. Over a period of 3 months of feeding, we obtained 1.4 kilos of zinc bornylglucuronide. The yield of glucuronic acid and lactone obtained from the hydrolysis of bornylglucuronide was also very good. From 170 gm. of glycoside, 70 gm. of a mixture of lactone and acid were obtained.

\textit{Preparation of Glucuron}—60 gm. of the mixture of glucuron and glucuronic acid obtained from the hydrolysis of bornylglucuronide, were very finely pulverized. 600 cc. of glacial acetic acid were placed in a 1 liter wide mouthed Erlenmeyer flask, and heated to boiling over an electric hot-plate. The boiling acetic acid was mechanically stirred, and the pulverized mixture of acid and lactone was added as rapidly as its solution permitted. After the material was entirely dissolved, the solution was cooled as quickly as possible. The lactone crystallized promptly from the solvent. After standing in the ice chamber overnight, the lactone was finally separated and washed with ether to remove acetic acid.

\(^1\) Just before precipitating the glycoside as its zinc salt it is advantageous to heat the urine filtrate to 70\(^\circ\) rather than to the boiling point.
52 gm. of lactone were recovered, a yield of 89 per cent (calculated on the basis of the original mixture containing 75 per cent lactone and 25 per cent acid).

In order to obtain a glistening snow-white product, it is important to dissolve the material as rapidly as is possible, and to cool the acetic acid solution immediately after solution takes place. If the solution of glucuron remains hot for any length of time, an impure dark product is obtained. The entire operation should not require more than a few minutes, if the acid-lactone mixture has been properly pulverized. The sample of glucuron thus secured melts at 178° (corrected). The best solvent for recrystallization purposes is methyl alcohol, and the product thus secured melts at 180° (corrected) with decomposition. The melting point is not very sharp. In both instances a preliminary softening and slight darkening is to be observed.

The rotation of the lactone in water, after recrystallization from methyl alcohol, is

\[ [\alpha]^2_b = \frac{+0.71 \times 100}{2 \times 1.914} = +18.55° \]

The solution shows no mutarotation.

Preparation of Pure Glucuronic Acid from Glucuron—Quick has prepared glucuronic acid of 99 per cent purity by extracting with alcohol the mixture of acid and lactone obtained from the hydrolysis of bornylglucuronide. This method, while effective, is unsatisfactory, for it gives low yields of acid. Glucuronic acid and glucuron are both extremely sensitive to the hydroxyl ion. We have found, however, that glucuron can be converted to the barium salt of glucuronic acid under certain conditions. The procedure was as follows:

11.4 gm. of pure glucuron were dissolved in 200 cc. of water. 10 gm. (slightly less than the theoretical quantity) of carbonate-free crystalline barium hydroxide (Ba(OH)_2·8H_2O) were dissolved in 300 cc. of water. A few drops of phenolphthalein were added and the solution of glucuron was stirred. The solution of barium hydroxide was added drop by drop from a separatory funnel at such a rate that at no time did the solution of glucuron become definitely alkaline to phenolphthalein. The solution of glucuron remained clear and practically colorless if the addition of base was
not too rapid. After all of the barium hydroxide had been added (1½ hours), the solution was concentrated to 75 ce. in vacuo, decolorized with a little norit, filtered, and then poured into 10 volumes of chilled methyl or ethyl alcohol. The barium salt of glucuronic acid was isolated, yielding 22 gm., or 97 per cent of the theoretical amount. The compound was then dissolved in 150 cc. of water, cooled to 0°, and decomposed by adding the proper amount of 3 N sulfuric acid. The barium sulfate was separated by centrifugation at 0° and was washed with cold water. The solution of glucuronic acid was carefully concentrated in vacuo to a thick syrup. The temperature within the distilling flask was maintained at 16°. The syrup was dissolved in absolute ethyl alcohol, and again evaporated nearly to dryness. A second solution with ethyl alcohol could not be effected because the acid crystallized out. The flask containing the acid was placed in the ice box for 2 days, and the free glucuronic acid was separated. 11.2 gm. were recovered. An appreciable amount of material could be recovered from the mother liquors, though this product contained considerable lactone.

When titrated with 0.025 N sodium hydroxide at 0°, with phenolphthalein as indicator, the acid thus prepared showed a purity of 99.1 per cent. It has been found that in the presence of lactone glucuronic acid cannot be titrated accurately with 0.1 N NaOH at room temperature, for the lactone is rapidly split by the sodium hydroxide at 20°. Even at 0°, when titrating with 0.025 N alkali, the end-point is transitory, and lasts only a few seconds, if appreciable quantities of lactone are present.

Pure glucuronic acid was prepared by recrystallization of the above product from 85 per cent ethyl alcohol, which seems to be the best available solvent for this purpose. The acid thus finally isolated, when titrated with 0.025 N NaOH, proved to be exactly 100 per cent pure.

\[
[a]_D = \frac{+0.54 \times 100}{2 \times 1.6820} = +16.05° \text{ (in water, taken 3 minutes after complete solution)}
\]

Solutions of the acid mutarotate rapidly and reach a constant value of +36° after 3 hours. The substance melts at 165° (corrected). It is to be noted that Ehrlich and Rehorst (6), who first
isolated crystalline glucuronic acid, reported a melting point of 154°.

**Oxidation of Glucuronic Acid and Glucuron by Sodium Hypoiodite (7)**—Glucuronic acid and glucuron should both be oxidized by sodium hypoiodite to the sodium salt of saccharic acid according to the equations:

\[(1) \text{HOOC—(CHOH)}_4\text{-CHO} + 4\text{NaOH} = \text{NaOOC—(CHOH)}_4\text{-CO-ONa} + 2\text{NaI} + 3\text{H}_2\text{O}\]

\[(2) \text{Cy-(CHOH)}_4\text{-CH-CHO} + 12\text{NaOH} = \text{NaOOC—(CHOH)}_4\text{-CO-ONa} + 2\text{NaI} + 2\text{H}_2\text{O}\]

In Equation 2 oxidation and hydrolysis of the lactone take place simultaneously. It was the purpose of these experiments to ascertain whether glucuronic acid and its lactone, when treated with sodium hypoiodite, would be stoichiometrically oxidized as is glucose. Nelson and Cretcher (8) found that the lactone of mannuronic acid was not.

Standard solutions of equimolar concentrations of glucose, glucuronic acid, and glucuron were made by dissolving 0.4500 gm. of pure and dry crystalline glucose, 0.4850 gm. of pure glucuronic acid, and 0.4400 gm. of glucuron in water and diluting to 100 cc. The glucuronic acid was, of course, first neutralized with the equivalent quantity of sodium bicarbonate. Samples were then pipetted off. Calibrated pipettes were used. The analyses were carried out in the usual way (9); the proper concentration of water was maintained when the smaller samples were analyzed. When samples of glucuron were analyzed, the equivalent quantity of 0.1 N NaOH was added from a burette, in addition to the alkali necessary for analysis. The results of the analytical data are given in Table I for glucuronic acid and glucuron. The results for glucose are not tabulated because analyses of 100 ± 0.2 per cent were obtained in each instance.

It is extremely interesting to observe that glucuronic acid itself is stoichiometrically oxidized to saccharic acid by means of hypoiodite, whereas in the case of its lactone, the oxidation progresses beyond that point. We have pointed out that the lactone is unstable to alkali at room temperature. The reaction between
glucuron, hypiodite, and sodium hydroxide must necessarily be complex, for not only is the aldehyde group of glucuron being oxidized to the carboxylic acid, but the lactone ring in the newly formed derivative, and in glucuron itself, is constantly being opened. It would seem, then, that at just this moment the compound is more subject to the action of the hypiodite and therefore might yield results which are not stoichiometric.

Determination of Reducing Value of Glucuronic Acid and Glucuron by Method of Bertrand (10)—The reducing value of samples of glucuronic acid and glucuron were determined by the method of Bertrand. A check on the analytical procedure was made by using equivalent quantities of optically pure crystalline glucose.

<table>
<thead>
<tr>
<th>Sample analyzed</th>
<th>0.1 N iodine necessary for stoichiometric oxidation</th>
<th>0.1 N iodine utilized in oxidation</th>
<th>Oxidation as calculated from iodine consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronic acid</td>
<td>Glucuron</td>
<td>cc.</td>
<td>per cent</td>
</tr>
<tr>
<td>145.5 mg.</td>
<td>15.00 cc.</td>
<td>14.97 cc.</td>
<td>99.8</td>
</tr>
<tr>
<td>97.0 mg.</td>
<td>10.00 cc.</td>
<td>10.00 cc.</td>
<td>100.0</td>
</tr>
<tr>
<td>48.5 mg.</td>
<td>5.00 cc.</td>
<td>5.01 cc.</td>
<td>100.2</td>
</tr>
<tr>
<td>132 mg.</td>
<td>15.00 cc.</td>
<td>15.96 cc.</td>
<td>106.4</td>
</tr>
<tr>
<td>88 mg.</td>
<td>10.00 cc.</td>
<td>10.78 cc.</td>
<td>107.8</td>
</tr>
<tr>
<td>44 mg.</td>
<td>5.00 cc.</td>
<td>5.35 cc.</td>
<td>107.0</td>
</tr>
</tbody>
</table>

We chose weights of glucose of 100, 75, 50, and 25 mg., and determined the mg. of copper reduced by these samples. The values obtained were almost identical with those given in Bertrand's tables, so we have tabulated the values as given by him. The weights of glucuronic acid and of glucuron equivalent to these arbitrarily chosen samples of glucose are given in Table II. Their reducing values were likewise determined.

We have found that glucuronic acid and its lactone have the same reducing value in Fehling's solution, but that equivalent quantities of these two derivatives have a reducing value slightly less than pure glucose itself. In quantities of 50 mg. or less the difference in reducing values is so slight as to be scarcely measurable by the macromethod of Bertrand, but with equivalent quan-
tities greater than these, the differences are readily detectable by this excellent titrimetric method.

Determination of Reducing Values of Glucose, Glucuron, and Glucuronic Acid by Micromethod of Shaffer and Hartmann (11)—Our experience with the micromethod of Shaffer and Hartmann has been that with each new lot of copper reagent it is advisable to determine the reducing value of glucose anew, rather than ac-

TABLE II
Reduction Values of Equimolar Quantities of Glucose, Glucuronic Acid, and Glucuron by Various Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample analyzed</th>
<th>Copper reduced by</th>
<th>Ratio of mm copper reduced to mm sample analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>Glucuronic acid</td>
<td>Glucuron</td>
</tr>
<tr>
<td>Bertrand</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>107.8</td>
<td>97.8</td>
<td>177.8</td>
</tr>
<tr>
<td>75</td>
<td>90.8</td>
<td>73.3</td>
<td>137.9</td>
</tr>
<tr>
<td>50</td>
<td>53.9</td>
<td>48.9</td>
<td>95.4</td>
</tr>
<tr>
<td>25</td>
<td>26.9</td>
<td>24.5</td>
<td>49.6</td>
</tr>
<tr>
<td>Shaffer-Hartmann micro-</td>
<td>1.350</td>
<td>1.455</td>
<td>3.00</td>
</tr>
<tr>
<td>method</td>
<td>1.080</td>
<td>1.164</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td>0.810</td>
<td>0.873</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>0.540</td>
<td>0.582</td>
<td>0.99</td>
</tr>
<tr>
<td>Shaffer-Hartmann macro-</td>
<td>100</td>
<td>97.8</td>
<td>218.5</td>
</tr>
<tr>
<td>method</td>
<td>75</td>
<td>80.8</td>
<td>165.9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>53.9</td>
<td>110.4</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>26.9</td>
<td>54.7</td>
</tr>
</tbody>
</table>

cept the values tabulated by the authors. We have determined the reducing values of glucose, glucuron, and glucuronic acid with a single lot of reagent, and the results are given in Table II. In these analyses, 5 cc. of micorcagent were used, and 5 cc. of sugar solution. The reduction was carried out in covered Pyrex test-tubes, 20 × 200 mm., the tubes were heated for 15 minutes in a boiling water bath, and the “cuprous” titration was performed.
The quantities of glucose used were 1.35, 1.08, 0.81, and 0.54 mg. The equivalent quantities of glucuronic acid and of glucuron are tabulated.

From the results in Table II, it is seen that glucuronic acid and glucuron both reduce considerably less copper per mol than does glucose. It is also of interest to note that glucuron reduces even less than does its homologue, the free acid. It has been found that aldobionic acids (Heidelberger and Goebel (1)), on the other hand, have approximately the same equivalent reducing value as has glucose, when determined by this method.

Reducing Values of Glucose, Glucuronic Acid, and Glucuron as Determined by Macromethod of Shaffer and Hartmann—The reducing values of the three derivatives were determined by the macromethod of Shaffer and Hartmann. Here again, it was necessary to perform control experiments on equivalent quantities of glucose, because the copper values which we found for this sugar were slightly higher than those given by Shaffer and Hartmann. The results are also given in Table II.

From this data, it is to be seen that glucose has a slightly higher reducing value per mol than glucuronic acid, and that the latter in turn reduces slightly more copper than does its lactone, glucuron. These differences are not as striking as they are in the micromethod of Shaffer and Hartmann.

In conclusion, we must point out that there are statements in the literature which refer to glucuronic acid as a powerful reducing agent—so powerful, in fact, that it will reduce Fehling's solution at room temperature. This is by no means the case when one is dealing with pure glucuronic acid. The substance will, of course, reduce Fehling’s solution at room temperature on prolonged standing, but apparently to no greater extent than does glucose. In reality, glucuronic acid and its lactone are, if anything, weaker reducing agents than glucose.

**SUMMARY**

1. A method for the preparation of glucuronic acid from its lactone, glucuron, has been given.

2. The relative reducing values of glucuronic acid and glucuron have been studied.
BIBLIOGRAPHY

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